MULTIPLE BIOCHEMICAL MARKERS FOR BREAST CANCER

by

Yu Xiongwen

A thesis submitted as partial fulfillment of the requirement for the degree of Master of Science

March, 1998

Department of Chemical Pathology

Faculty of Medicine

The Chinese University of Hong Kong

UL


香港中文大學
圖書館系統
15 JUL 1999
UNIVERSITY
LIBRARY SYSTEM
THE CHINESE UNIVERSITY OF HONG KONG

ACKNOWLEDGMENTS

I gratefully acknowledge my sincere appreciation to the support and guidance provided by my supervisor, Dr. C P Pang, during the course of this study and in the preparation of the dissertation.

I would like to thank Professor Walter King, Department of Surgery, for patients' recruitment and blood specimens collection.

I would like to thank Mr. Eric Law, scientific officer of the Department of Chemical Pathology, for his effort to arrange samples and clinical information of the study subjects involved in this study.

My thanks also go to Ms. En Fong Wong, Ms. Karen, Mr. Simon, Ms Alice Liu, Ms Iris, for their help in my work.

Last but not the least, I must thank Prof. NM Hjelm, Chairman of the Department of Chemical Pathology, for his support and consideration throughout the Msc course.

ABSTRACT

The potential usefulness of CA 15-3, TPA, and apolipoprotein(a) in diagnosis and monitoring of breast cancer patients was evaluated in 188 female patients with histologically confirmed breast cancer. Among them 60 have blood samples collected before surgery and about 2 weeks after surgery and 8 have follow-up blood samples. Also recruited were 97 normal women and 15 female patients with benign breast disease as controls. Plasma CA 15-3, TPA and apolipoprotein(a) were measured using MEIA, ELASA and RIA respectively. Both plasma CA 15-3 and TPA levels, not Apo(a), were shown elevated in breast cancer. The cut-off levels for CA 15-3 and TPA at 22U/ml and 1.40 ng/ml, respectively, gave 95% specificity, at this cut-off, the diagnosis sensitivity of CA 15-3 and TPA was 18% and 20% respectively. Using ROC analysis, selected cut-off value 15U/ml for CA 15-3 and 1.20 ng/ml for TPA, the sensitivity were 46% and 25% with specificity 78% and 89% respectively. Since there is no correlation between CA 15-3 and TPA, the combination use of both markers gave sensitivity and specificity of 53% and 75% respectively. The CA 15-3 and /or TPA are not useful for screening and diagnosis breast cancer due to its low sensitivity. The proportion and magnitude of changes in CA 15-3 levels after surgical treatment were higher than that of TPA. In conjunction with other diagnostic tests, both markers in combination may be useful for diagnosis and monitoring of breast cancer. Their role in the follow-up and prognosis of breast cancer patients is a subject for further investigation.

摘要

应用 MEIA、ELASA 和 RIA 的分析方法，对 188 例乳腺癌患者、15 例良性乳腺病患者及 97 例正常对照者的血浆中 CA 15-3、TPA 和 Apo(a)浓度进行研究。其中对 60 例乳腺癌患者作手术前后对比研究。**结果：**乳腺癌患者血浆中 CA 15-3、TPA 浓度显著升高，Apo(a)无显著性差异。CA 15-3、TPA 断点分别取 22U/ml、1.40ng/ml, 特异度为 95%,其灵敏度分别为 18%、20% 。应用 ROC 方法分析，CA 15-3： 15 U/ml, 灵敏度 46%、特异度 78%; TPA ： 1.20 ng/ml, 灵敏度 25% 特异度 89% 。由于 CA 15-3 和 TPA 无相关，联合应用其灵敏度和特异度分别为 53%、75% 。手术后血浆水平变化 CA15-3 大于 TPA。**结果表明：**由于灵敏度偏低，血浆 CA 15-3 和 TPA 不适合单独用于筛查及诊断乳腺癌。结合其它方法，可帮助诊断及监控乳腺癌。其在乳腺癌预后的作用有待进一步研究。

LIST OF TABLES

LIST OF FIGURES

TABLE OF CONTENTS

Chapter 1 Introduction

1.1 Tumor Marker

1.1.1 General concept of tumor markers

Tumor marker can be defined as a substance present in or produced by a tumor or by the tumor's host in response to the tumor's presence that can be used to differentiate a tumor from normal tissue or to determine the presence of a tumor based on measurement in the blood or secretions. Such a substance can be found in cells, tissues, or body fluids. It can be measured qualitatively or quantitatively by chemical, immunological, or molecular biological methods to determine the presence of a cancer (Sell, 1992).

It does not necessarily have to be tumor-specific, but may be a substance present in blood or urine or expressed at the cell surface in larger quantities by malignant cells or their environs than by their normal counterparts, and may thus represent a relative rather than an absolute marker of malignancy. Tumor markers that are produced within the tumor, whether by malignant or stromal cells, are said to be

tumor-derived, whereas those markers produced by non-malignant cells as a result of disturbance by the tumor are said to be tumor-associated.

The first tumor marker discovered was the Bence-Jones protein (Bence-Jones, 1867). Since its discovery in 1847 by precipitation of a protein in acidified boiled urine, it has been a diagnostic test for multiple myeloma. The general application of tumor markers for monitoring cancer patients started until the discovery of α-fetoprotein (AFP) in 1963 (Abelev et al, 1963) and carcinoembryonic (CEA) in 1965 (Gold et al, 1965). A brief history of the development of tumor marker research is shown in ***Table 1-1***. (Roulston et al, 1993).

1.1.2 Application of tumor markers

(a). Screening for primary disease

A tumor marker with excellent specificity and sensitivity may be use to diagnose primary disease before it is suspected clinically. Such a marker would significantly improve the chances of successful therapy, since the tumor could be treated at an early stage or before metastases have developed.

(b). Diagnosis of primary disease

A tumor marker could be used to establish the diagnosis of malignancy in a patient with symptoms, preferably in the early stages of the evolution of the tumor.

Table 1-1 Some milestones in tumor marker research

Year	Author(s)	Contribution
1846	Bence-jones	Bence-jones protein
1928	Brown	Ectopic hormone syndrome
1930	Zondek	Human chorionic gonadotrophin (hCG)
1932	Cushing	Adrenocorticotropin (ACTH)
1933	Gutmann & Gutmann	Prostatic acid phosphatase (PAP)
1949	Oh-Uti	Deletion of blood group antigens
1959	Market	Isoenzymes
1960	Newell	Philadelphia chromosome
1963	Abelev	Alphafetoprotein (AFP)
1965	Gold & Freeman	Carcinoembryonic antigen (CEA)
1969	Heubner & Todard	Oncogenes
1975	Kohler & Milstein	Monoclonal antibodies
1980	Cooper, Weinberg & Bishop	Oncogene probes and transfection
1985	Harris, Sager & Knudson	Suppressor gene

(Modified from J.E. Roulston, R.C.F. Leonard: Serological Tumour Markers. 1993,pp3-5)

(c). Prognosis of treatment

The tumor marker concentration at the time of diagnoses may be used to predict the likely clinical outcome. To be of value in prognosis, the tumor marker concentration should correlate closely with tumor size and / or activity, so that a modest elevation means a small localized tumor, whilst a greater elevation suggests bulky disseminated malignancy or an aggressive tumor.

(d). Monitoring of treatment

The concentration of a particular tumor marker in a tumor marker positive patient may be used as a crude index of the size and/or activity of the tumor. The effectiveness of the therapy used to treat the tumor, whether it be surgery, chemotherapy, radiotherapy or a combination of these, may be monitored by measurement of appropriate tumor markers. Effective therapy to reduce the size /activity of the tumor will result in a corresponding fall in the plasma concentration of the tumor marker.

(e). Indication of recurrence

One of the most valuable functions of a tumor marker is to provide an early indication of tumor marker recurrence. Thus, there is a great merit in continuing to monitor a patient who was tumor marker positive, even after the concentration of that marker has stabilized, normalized or become undetectable following successful therapy. A further significant rise in the concentration of the tumor marker may provide the first evidence of recurrence and so allow for providence of recurrence and so allow for prompt and effective second line therapy.

1.1.3. Limitation of tumor markers

An ideal marker should be:

- stable and accurately measurable even in low concentrations
- present in readily accessible samples, such as blood and urine
- secreted exclusively by tumor cells of a specific morphological type
- quantitatively correlated with tumor burden and progress
- measurable even when produced by a barely detectable tumor mass
- differentiate benign and malignant or metastatic tumors.

(Modified from C P Pang (1995) Tumor markers. Study Guide: 2-6)

At the moment there is no tumor marker of proven value for early detection of breast cancer, no single marker of 100% sensitivity and specificity. There are some of prognostic indicating value and indication of recurrence but so far no single marker is absolutely satisfactory. An ideal tumor marker does not exit but there are tumor markers having established clinical value. AFP (α-Fetoprotein) is a marker for hepatocellular and germ cell (nonseminoma) carcinoma. (Taketa et al, 1992). PSA (Prostate-Specific Antigen) is one of the most promising tumor markers. It is an organ-specific tumor marker, can be used to early detection, staging and monitoring of prostate cancer.(Chu, 1992; Oesterling, 1991).

1.2 Breast Cancer

1.2.1 Incidence in Hong Kong women

Breast cancer has been recognized as a life-threatening disease since the time of the ancient Egyptians. Early reports of breast cancer can be traced to the 1930's (Baum M., 1982). Breast cancer is the most frequent type of cancer among women, accounting for approximately 4% of all deaths, 20% of all cancer deaths, and 25% of all cases of cancer in Western Europe and North American (Meden et al., 1995).

In Hong Kong, breast cancer is now the second most common cancer and the second leading cause of cancer death in Hong Kong women. The annual incidence of breast cancer has increased to more than 1000 cases, which is three times more than the figure observed 30 years ago. In 1991, there were 1133 new cases of lung cancer and 1106 new cases of breast cancer in women. In the same year, 881 women died from lung cancer and 333 women died from breast cancer (1993 Report, Department Health, Hong Kong Government). Table 1-2 showed that approximately 50% of the breast cancer presented at an advanced stage in 1960s and 1970s. In the latter part of the 1980s, there were nearly 20% of the breast cancer present at an advanced stage and 60% at stage II. (Cheung, 1992)***(Table 1-2)***

Table 1-2 Staging at presentation in different time periods in Hong Kong.

	1966-75	**1976-85**	**1986-90**
	%	**%**	**%**
In-situ cancer	0	0	2.1
Stage I	6.1	6.1	14.9
Stage II	42.0	47.8	62.4
Stage III	45.7	37.8	19.6
Stage IV	6.1	8.3	1.0

(Modified from Polly S Y Cheung (1992) Breast cancer in Hong Kong- the need for early detection.J Hong Kong Med Assoc 4:248-252.)

1.2.2 Need for early diagnosis and prognosis

Breast cancer affects about 7% of all women in the western world and it is a leading cause of cancer mortality (Barak et al, 1990). The majority of patients with breast cancer will develop a recurrent and progressing disease (Harris et al, 1985) which requires systemic treatment to alleviate the symptoms or, occasionally, to prolong the patient's survival (Veach et al, 1987). The number of fatal cases could be reduced by 90% with better early detection and treatment modalities (Schwartz et al., 1992). Current screening procedures include breast self -examination (BSE), mammography, and clinical examination. The mortality rate in women who participate in screening programs is 50% lower at 5 years than for women who have mot been screened (Shapiro et al, 1985). In part as a result of the high rate of morbidity associated with this cancer, a great deal of effort has been exerted to identify markers useful for early diagnosis and patient follow-up. However, it is a fact that the treatment outcome depends upon the tumor burden at the beginning of therapy: the smaller the tumor burden, the more effective the treatment. At present, screening techniques such as chest X-ray, ultrasonography, bone scanning and skeletal surveys are of limited value in the detection of occult metastases. Unfortunately there are also no effective immunological or biochemical tests available to determine the presence of a small tumor mass.

1.3 Markers for Breast Cancer

1.3.1 Usefulness of tumor marker for breast cancer.

Breast cancer remains one of the most common lethal malignancies in the western word. Of the epithelial malignancies, it is arguably the one for which tumor markers might have the greatest potential. For example, it is now clear from the results of well-performed randomized trials that screening and early treatment of breast cancer (including both primary and adjuvant systemic therapy) are superior to later treatment (Early 1992; Eddy 1989; Nyström et al., 1993). However, not all of the population stands to benefit from these maneuvers, and effective use of tumor markers might permit the application of screening and treatment more efficiently (Early 1992; Eddy et al., 1988; Fletcher et al., 1992). Moreover, patients with established breast cancers may benefit from several different local and systemic therapies, each with various efficacies and toxicities (mastectomy, lumpectomy, radiation therapies, endocrine therapy, and chemotherapy).(Henderson et al.,1990) Tumor marker might permit selection of the most appropriate treatments for the individual patient based on her particular situation .***(Table 1-3,1-4).***

Table 1-3 Potential utilities of tumor markers for breast cancer

Setting	**Potential utilities**
Determine risk	Germ line genes in normal tissue
Screening	Circulating antigens
	Genes and/or antigens in nipple aspirate, breast biopsy, fine needle aspirate
Differential diagnosis	Tissue markers of hematologic vs. mesenchymal vs. epithelial organs
	Tissue markers of separate epithelial organs
	Circulating markers specific to separate epithelial organs
Prognosis	Genes or antigens in primary breast cancer tissue
	Genes or antigens in sccult, distant metastases
	Circulating antigens related to tumor burden or biology
Prediction of response to:	
Endocrine therapy	Estrogen receptor / Progesterone receptor
Chemotherapy	Tissue-based marker
Novel therapies	Tissue, circulating markers
Prediction of impending relapse	Circulating antigens
Monitoring disease course	Circulating markers

(Modified from Daniel F. Hayes, Tumor markers for breast cancer: Current utilities and future prospects. Hematology / Oncology Clinics of North America. Vol 8. 3,1994.)

Table1-4 Cancer markers that have been used for investigation of breast cancer.

Name	Nature	Normal limit	Use*			Sensitivity %	Specificity %
			D	P	M		
CEA(carcinoembryonic antigen	200 kDa glycoprotein	2.5-5.0 μg/ml	+	++	+++	42-96	10-90
TAG-72	Sialyl Tn	4-7 U/ml	++	++		9—72	97
CA 15-3	High mol wt glycoprotein	35 U/ml	+	++	+++	88—97	30—90
CA 19-9	Sialyated Lewis XA	37 U/ml	+	++	++	33—89	89—97
CA 549	Glycoprotein	13U/ml	+	+	++	78.1	97.4
MCA	350 kDa glycoprotein	11 U/ml	+	++	+++	20—80	84—94
TPA	Cytokeratins 8, 18 and 19	85 U/ml		+	+++	67—80	75
Cathepsin D	cysteine proteases			+	++		
Sp2	Sp2 protein	14 U/ml					
Estrogen progesterone receptors	intracellular polypeptides receptors	10,000/cell		+++	+		
c-erbB-2	gene		++	+++			
p53	tumor suppressor gene		+	+++			

* (D: Diagnosis; P: Prognosis; M: Monitoring)

(Modified from: Serological Cancer Markers. Stewart Sell, Ed. Totowa, NJ, The Humana Press, 1992)

1.3.2 Some tumor markers for breast cancer.

Carcinoembryonic Antigen

Carcinoembryonic antigen (CEA) is a large family of related cell-surface glycoproteins. The CEA family consists of about 10 genes located on chromosome 19. Up to 36 different glycoproteins have been identified in the CEA family. CEA is a glycoprotein with a molecular mass of 150 to 300 kD and contains 45 to 55% carbohydrate. It is a single polypeptide chain consisting of 641 amino acids, with lysine in the N-terminal. CEA was originally introduced as a marker for colonic cancer (Gold et al, 1965) but it has later proved to be a universal marker for adenocarcinomas including breast cancer (Laurence et al, 1972)

Estrogen receptors and progesterone receptors.

The estrogen receptors and progesterone receptors are intracellular receptors that are measured directly in tumor tissue. These receptors are polypeptides that bind their respective hormones, translocate to the nucleus,(Greene et al.,1984; Green et al.,1987) and induce specific gene expression.

The estrogen receptor and progesterone receptor might be able to: (a) predict a response to specific therapies for metastatic disease: (b) predict the success of adjuvant hormonal therapy: and (c) assess the prognosis of a specific tumor to metastasize.

c-erbB-2

The c-erbB-2 (HER-2/neu) gene encodes a transmembrane tyrosine kinase that is the receptor for a family of peptide hormones (Gullick, 1990). Various methods have been used to measure c-erbB-2 gene and its product. These include direct measurement of gene amplification, mRNA level, and protein expression.

Amplification of c-erbB-2 is found in breast, ovarian, and gastrointestinal tumors. In breast cancer, it appears to be as useful a prognostic indicator of overall survival as tumor size or estrogen and progesterone receptor expression but not as good as the number of lymph nodes involved in metastases (Slamon et al, 1989)

P53

p53 is a tumor suppressor gene on the shore arm of chromosome 17 that encodes a protein that is important in the regulation of cell division. The p53 gene product appears to regulate transcription of several other genes. There is evidence that the gene product is important in preventing the division of cells containing damaged DNA (Levine et al, 1991; Finlay et al, 1989).

p53 gene deletion is a frequent event along with other molecular abnormalities in colorectal carcinogenesis. Allelic deletions of p53 occur in approximately 60% of human breast cancer (Prosser er al, 1990). With immunohistochemistry, p53 expression is detectable in 36% to 46% of clinical breast malignancies (Cattoretti et al, 1988; Ostrowski et al, 1991). p53 has also been associated with erbB-2 positivity (Barbareschi et al, 1992) In short-term follow-up , p53 shows

a trend toward poorer prognosis (Ostrowski et al, 1991)

Cathepsin-D

Cathepsin D, reported originally as a 52-kD proenzyme secreted form (Capony et al, 1982), is an estrogen-induced lysosomal protease that is abnormally present in breast cancer cell.(Copony, et al, 1989)

This proteolytic enzyme can react against basement membranes. Cathepsin-D also has mitogenic activity on MCF-7 cells that are estrogen depleted. Further studies showed that cathepsin-D was relatively low in resting mammary cells but was elevated in malignant and benign proliferative breast disease. These findings raised the suspicion that the cathepsin-D could both promote abnormal growth of cells as well as contribute to the metastatic potential of malignant cells through its disruption of the basement membrane and therefore might be a marker for a poor prognosis in breast cancer.(Tandon et al., 1990). Several studies have shown that high cathepsin D levels in extracts of tumor tissue are associated with poor prognosis. (Spyratos et al, 1989; Tandon et al, 1990; Thorpe et al, 1989)

CA 549

Chemical characterization of this antigen has shown it to be a high molecular weight acidic glycoprotein. CA 549 can be separated into species with molecular masses of 400 and 512 kd. One monoclonal antibody, a murine IgG1 termed BC4E 549, was raised by immunizing mice with partially purified membrane preparations from T417 human breast tumor cell line. The other antibody, BC4N 154 (a murine IgM),

was developed against human milk fat globule membranes (Bray et al, 1987). CA 549 is not useful in detecting early breast cancer. CA 549 is better than CEA at identifying active breast cancer. In the monitoring of advanced breast cancer patients, CA 549 correlates with disease progression and regression and helps detect metastases (Chan et al, 1988).

MCA

MCA is referred to as mucinous carcinoma associated antigen. (Browning et al., 1988). MCA was identified on the surface of a breast carcinoma cell line by the monoclonal antibody b-12. I t is a marker for breast cancer (Bombardieri et al, 1992). Preliminary data show that level of MCA in sera correlate well with those of CA 15-3. MCA seems to be marginally superior to CA 15-3 in the follow-up of patients with breast cancer but again this remains to be confirmed (Browning et al., 1988)

MSA

MSA or mammary serum antigen is defined by reaction with the monoclonal antibody 3EI.2. (Stacker et al., 1985). Preliminary data suggest that MSA may be a more sensitive marker for breast cancer than CA 15-3. There was study shown that MSA was elevated in 68% of patient with stage I and II breast cancer compared to only 3% for CA 15-3(Sacks et al. 1987). These findings however, remain to be confirmed.

1.4 Selective Markers for Breast Cancer in This Study.

1.4.1 TPA

Tissue polypeptide antigen (TPA) was originally described by Björklund and Björklund (1957). It was isolated from the insoluble residues of pooled tumors. Horse antiserum against the protein recognized both soluble form of TPA in human sera and body fluids, and an in soluble form in human tissues. Originally TPA was thought to be a tumor marker and, more recently, a proliferation marker (Björklund, 1980).

During the 1980's, a unique type of proteins, intermediate filaments, was discovered in epithelial cells. Intermediate filaments can be subdivided into five groups: cytokeratins, desmin, vimentin, GFAP (glial fibrillary acidic protein) and neurofilaments(Lazarides E 1982; Virtanen L et al, 1981). The cytokeratins, of which 20 have been isolated from different epithelial tissues, are further subdivided into type I (cytokeratins 9-19 and perhaps 20) and type II (cytokeratins 1-8)(Lazarides, 1980; 1982; Moll et al, 1990;Steinart et al, 1985;Weber et al, 1984). It is now widely accepted that, for a normal formation of intermediate filaments two different cytokeratins are necessary, one type I and one type II, which form a pair. Cytokeratins appear in different combinations in different epithelial tissues and in each cell type, there is one or more cytokeratin pairs (Moll et al, 1982; Quinlan

et al, 1985)

Cytokeratins are multigene-coded and the different subtypes are related to the type of epithelial cell, and the extent of cell differentiation and development in the tissue. The genes for cytokeratins 8 and 18 are both found on chromosome 12(Waseem et al, 1990a; 1990b).

Cytokeratin expression remains throughout all stages of epithelial malignancy. The smallest cytokeratin pair, 8 and 18, is found in large quantities in simple, ductal and glandular epithelium, pseudo stratified epithelium, transitionsl epithelium and carcinomas arising therefrom (Moll et al, 1982; Quinlan et al, 1985). Cytokeratins 8 and 18 have pI values of 6.1 and 5.7 respectively and molecular weights of 52kD and 45 kD respectively.

Cells in growing tumors and metastases release growth factors that stimulate endothelial cells in blood vessels to produce new blood vessels. The increased vascularization in the tumor results in further cell growth in parts of the tumor and an increased release of substances related to cell growth. This increased growth results in a blocking of blood vessels, cell death and cell lysis and release of proteolytic enzymes.

Proteolysis of cell material releases a spectrum of more or less degraded proteins, amongst them cytokeratins in their native form are

very insoluble and thus remain to a large extent in the necrotic regions of the tumor. There, along with the cell's intact cytokeratins, they can be visualized with immunohistological techniques. Solubilised fragments of cytokeratins can slowly leak out into the circulation.

It can be postulated that cytokines endogenously produced as a result of cell division in growing tumors can affect epithelial cells. This could cause not only a restructuring of the cytoskeleton but also a significant release of cytokeratin fragments into the circulation.

Serum cytokeratin fragment levels can be found in significantly higher concentrations in the serum of cancer patients than in normal individuals (Sundstrom et al, 1990). TPA (Tissue Polypeptide Antigen) has been described as a tumor associated antigen present in a variety of malignant tumors (Bjorklund et al, 1957). Numerous clinical investigations have shown a high frequency of elevated levels of TPA in the serum of patients with tumor progression. An even higher frequency of elevated values is found among patients with distant metastases.

A relationship between TPA and cytokeratins 8,18, and 19 has been demonstrated (Luning et al, 1983; Weber et al, 1984). Sequence identity between major fragments of TPA and cytokeratin 8 has been established as well as a high degree of homology (72%) between sequences of a TPA fragments and cytokeratin 18(Bahr et al, 1988; Leube et al, 1986; Redelius et al, 1980). It has been shown that

monoclonal antibodies against pertinent epitopes of cytokeratins 8,18 and 19 can replace, individually or in pairs, the polyclonal TPA antibody used in the TPA IRMA kit (Mellereck et al, 1990). An increased concentration of cytokeratin fragments in serum correlates with tumor progression.

In breast cancer, higher serum tissue polypeptide antigen levels were correlated with a poor prognosis (Gion[1] et al; 1990). Conversely, a high cytosol tissue polypeptide antigen level was shown to be a powerful, independent, favorable prognostic indicator (Gion[2] et al, 1990; 1993; 1994)

However, recent work has shown that antibodies used in the tissue polypeptide antigen assay strongly react with cytokeratins 8 and 19, which tissue polypeptide specific antigen (TPS) antibody strongly reacts with cytokeratin 18, but very weakly with cytokeratin 8 in the immunoblot assay (Bodenmuller et al, 1994).

1.4.2 CA15-3

Cancer Antigen 15-3

Monoclonal antibodies have recently been developed that detect circulating breast cancer associated antigen. CA15-3 assay values are defined by using the 115D8 and DF3 monoclonal antibodies.(Hayes DF, et al, 1986; Tobias et al, 1985).

The monoclonal antibody DF3, raised against a membrane enriched fraction of metastatic human breast carcinoma, react with epitopes

expressed by a family of high molecular weight glycoproteins designated as polymorphic epithelial mucins(PEMs)(Hilkens[1] et al, 1984; Hilkens[2] et al, 1984; Kufe et al, 1984; Taylor-Papadimitriou et al, 1988). This antigen is a mucin-like glycoprotein with a highly variable molecular weight of 300-450 kD (Sekine et al.,1985; Hayes et al.,1986). More recent research places the cause of its electrophoretic heterogeneity in the autosomal codominant expression of multiple alleles at one gene locus (Siddiqui et al., 1988). The gene for this molecule has been located on chromosome 1q. cDNA cloning indicates that the DF3 peptide core consists of a highly conserved 60-bp tandem repeat sequence. The polymorphism of the antigen is the result of different repeats in the peptide core. The DF3 antibody recognizes the epitope within this 20 amino acid repeating sequence of the peptide core. The recognition of the epitope is also affected by glycation.(Hayes et al.,1992) Details of biochemical analyses suggest a relationship of the DF3 antigen with the sialyloligosaccharides (Ochuchi et al.,1984) by the presumed involvement of a terminal N-acetyl-D-neuramic acid or a N-acetyl-glycosamine-residues on the development of the antigenic determinant (Sekine et al.,1985).The DF3 antigen can be found on the surface and in the cytosol of human breast cancer cells. The immunoperoxidase technique shows this DF3 antigen primarily in the apical border region of benign secretory epithelial cells of the breast, whereas it was observed the accumulating in the cytosol of less differentiated, malignant cancer cells (Kufe et al., 1984). The technique also showed cytoplasmic staining in 78% of breast cancers and in 8% of fibroadenomal or fibrocystic disease specimens (Kufe et

al., 1984; Lundi et al., 1985).

The second monoclonal antibody 115D8 raised against human milk-fat globule membranes (Hikens et al., 1984). It recognizes an antigenic determinant localized on the MAM-6 antigen, a very large glycoprotein of over 400 KD (Hikens et al., 1985). It has been shown, that many other malignancies, particularly tumors of the ovaries, the endometrium and the cervix,(Hikens et al.,1984) and some normal tissues like salivary and sweat glands,(Hageman er al., 1984) can produce large quantities of this antigen.

Studies have demonstrated that monoclonal antibody DF3 and 115D8 recognize distinct epitopes on the same antigen (Abe et al., 1987).

Research studies have indicated that CA 15-3 assay values are frequently elevated in patients with breast cancer.(Bon et al.,1990; Colomer et al.,1989; Dnistrian et al.,1991; Safi et al;1991;Silver et al.,1991;Tondini et al 1988). These studies have suggested that the CA 15-3 assay may be of clinical value for monitoring the response of patients undergoing therapy because increasing and decreasing value correlated with disease progression and regression, respectively. (Robertson et al., 1990;Hayes et al 1986;Silver et al., 1991). Additional published studies have suggested that increase CA 15-3 assay values in patients at risk for breast cancer recurrence after prime therapy may be indicative of recurrent disease before it can be detected clinically (Geraghty et al., 1992; Safi et al., 1991;Hilkens et al, 1986)

Elevations of CA 15-3 assay values have been reported in individuals with nonmalignant conditions such as cirrhosis, hepatitis, autoimmune disorkers and benign disease of the ovary and breast.(Bon et al.,1990;Colomer et al 1989). Non-mammary malignancies in which elevated CA 15-3 assay values have been reported include lung, colon, pancreatic, primary liver, ovarian, cervical and endometrial(Bon et al 1990;Colomer 1989) CA15-3 assay values are not elevated in most normal individuals(Bon et al 1990).

1.4.3 Apolipoprotein(a)

Lipoprotein(a) represents a family of lipoprotein particles varying in density between 1.05 and 1.10 g/ml and in size, owing to differences in protein-lipid ratio and protein composition. The protein moiety of Lp(a) consists of apolipoprotein B-100 linked to apolipoprotein(a) by one or two disulfide bridges. It is slightly larger in size than LDL with a lipid composition similar to LDL, except for a relatively higher content of triglycerides (Scanu et al, 1991).

Apolipoprotein(a), Apo(a), is a highly glycosylated protein linked by disulphide bridges to apolipoprotein B in the lipoprotein(a)(Lp(a)) particle and conferring the characteristic properties to the Lp(a) complex (Albers et al,1990). Apo(a) is formed by three different structural domains. One of the domains, called kringle 4, is present in multiple copies, the number of which varies and is genetically

determined, giving rise to different sizes of apo(a) and consequently Lp(a). Depending on the method used, six to 23 isoforms of apo(a) ranging from about 300 to 900 kD have been identified (Uterman et al,1989; Mbewu et al, 1990; Lackner et al, 1991; Kamboh et al, 1991). Most individuals have one or two Apo(a) isoforms, although in some subjects no Apo(a) band can be detected when analyzed in SDS-gel electrophoresis followed by immunoblotting (Albers et al, 1990).

Up to the present time, much interest has been focused on Lp(a) since it has been associated with an increased risk of cardiovascular disease, and recently it was shown to be increased in various types of cancer (Wright et al,1989). Alterations in plasma lipoprotein metabolism are a well-documented phenomenon associated with tumor growth in both animals and humans (Barclay et al, 1975). In fact, individual lipoproteins have been suggested as potential markers of malignant disease (Skipski et al, 1975; Wieczorek et al, 1985; Mountford et al, 1987). There was an association between Lp(a) and malignant disease in acute myeloblastic leukemia (Niendorf et al, 1992). This study was designed to investigate the association of Apo(a) with breast cancer and with the progression of the disease.

1.5 Objectives

1.5.1 To evaluate the analytical performance of the three commercially available tumor markers: CA 15-3, TPA and Apolipoprotein (a).

1.5.2 To study the potential clinical usefulness of the three tumor markers in diagnosis and monitoring patients with breast cancer.

Chapter 2 Materials and Methods

2.1 Materials

2.1.1 Patients and control subjects

From April 1993 to October 1997, 188 patients with breast cancer and 15 benign breast disease patients attending the breast clinic of Department of Surgery, Prince of Wales Hospital, entered this study. Patients' selection, based on clinical features and histopathological data, was carried out by clinical oncologists in the Department of clinical oncology, Princes of Wales Hospital. 97 healthy and disease-free voluntary staffs of the Prince of Wales Hospital were recruited to serve as the control group.

2.1.2 Sampling

About 20ml morning fasting blood was obtained by venipuncture from each patient and healthy volunteer. For the cancer patients, pre-operative blood collection was taken immediately after diagnosis, post-operative samples was taken 10 days after surgery and before

treatments such as chemotherapy or radiotherapy. 10mL of blood was transferred into a EDTA tubes and the remaining 10mL into a plain tube. After centrifugation, the plasma and serum were aliquoted into 1ml portions and stored at –70°C before analysis.

2.2 Methods

2.2.1 CA 15-3: Cancer Antigen 15-3

The IMx® CA 15-3 assay is a Microparticle Enzyme Immunoassay (MEIA) on IMx System for the quantitative measurement of CA 15-3 in human serum and plasma.(Manufactured by Abbott laboratories, Diagnostics division, Abbott park, IL 60064, U.S.A.)

Dilution specimen and 115D8 antibody (mouse, monoclonal) coated Microparticles are added to the incubation well of the reaction cell. During the incubation of this reaction mixture, the 115D8 reactive determinant in the specimen binds to the monoclonal antibody 115D8 coated Microparticles forming an antibody-antigen complex. An aliquot of the reaction mixture is transferred to the glass fiber matrix to which the microparticles are irreversibly bound. The matrix is washed to remove unbound materials. The DF3 antibody (mouse, monoclonal):Alkaline Phosphatase Conjugate is dispensed onto the matrix and binds to the antibody-antigen complex. The matrix is washed to remove unbound materials. And the substrate, 4-

Methylumbelliferyl phosphate, is added to the matrix and the rate of fluorescent product formation is measured by the MEIA optical assembly.

The CA15-3 antigen content (U/ml) is calculated using a standard curve generated by standard solutions provided in the kit.

2.2.2 New TPA (Tissue Polypeptide Antigen)

The IDeal Monoclonal TPA_{cyk} ELASA is a test for the determination the key epitopes of TPA (Tissue Polypeptide Antigen) related to cytokeratins 8 and 18.(Manufactured by Mercodia AB, Seminariegatan 29, S-752 28 Uppsala, Sweden) Using BIO-RAD model 1575 immunowash and EL340 System microtiter plate reader adjusted for wavelength for 450nm.

IDeal Monoclonal TPA_{cyk} ELASA is a solid phase sandwich assay. Specimens, standards and controls are incubated with a monoclonal anti-cytokeratin antibody mixture coated to wells, simultaneously with a HRP- labeled antibody. During the incubation, both the immobilised antibody and the HPR-labeled antibody bind to the cytokeratin fragments forming a sandwich. The wells are washed and a TMB substrate is added. The developed color is proportional to the concentration of the analyte.

Both the old polyclonal antibody-based tissue polypeptide antigen (TPA-P), which had been thoroughly studied in breast cancer, and the more recently available monoclonal antibody-based tissue polypeptide antigen (TPA-M) were used. Indeed, the latter tissue polypeptide antigen (TPA-M)assay presents two advantages in comparison to the old one: firstly, the cytokeratins recognized by the monoclonal antibody are well known, and secondly, the use of monoclonal antibodies should provide more reliable, stable and reproducible assay systems.

IDeal Monoclonal TPA_{cyk} differs from TPA-P by applying an assay with the monoclonal antibody instead of polyclonal antobodies as a tracer. According to Björklund, TPA has nothing to do with cytokeratins and is, in contrary to new TPA, only related to cell proliferation. More recent findings, however, show that new TPA antibodies bind to keratin 8 and 18 positive tissue as well as to keratin negative tissues (Oehr et al., 1991)

2.2.3 Apo (a): Apolipoprotein(a)

Mercodia Apo(a) RIA provides a method for the quantitative measurement of human apolipoprotein(a)(Manufacture by Mercrodia AB, Seminariegatan 29 S-752 28 Uppsala, Sweden) using Auto-Gamma® 5000 Series Gamma Counting Systems.

Mercodia Apo(a) RIA is a solid phase two-side immunoradiometric assay. It is based on the direct sandwich technique in which two

monoclonal antibodies are directed against separate antigenic determinants on the apolipoprotein(a) molecule. During incubation apolipoprotein(a) in the sample reacts with 125-I-anti-apolipoprotein(a) antibodies and anti-apolipoprotein(a) antibodies bound to Sepharose® particles. The formed anti-antigen complex is separated from excess tracer by addition of Decanting suspension followed by centrifugation and decanting. The radioactivity in the pellet is directly proportional to the concentration of apolipoprotein(a) in the sample.

The kit is calibrated against a highly purified, fully validated, commercial Lp(a) preparation. The concentration of Apo(a) is expressed in U/L. It is not possible to express the concentration of Apo(a) in mass units as there are at least six different isoforms described with molecular weights varying from approximately 300 to 900 kD(Uterman 1989; MBewu et al.,1990; Lackner et al.,1991; Kamboth et al.,1991). Thus each patient sample will contain different proportions of isoforms. Therefore no conversion factor can be given between Units of Apo(a) and milligrams of Apo(a).

2.3 Statistical Methods

The predictive value model has been applied by a number of investigators for evaluating the clinical performance and effectiveness of clinical laboratory tests. Sensitivity, specificity, predictive value and total accuracy define a laboratory test's diagnostic accuracy (Gail,

1979). Sensitivity indicates the frequency of positive test results in patients with a particular disease; specificity indicates the frequency of negative test results in patients without that disease. The predictive value of a positive test result indicates the frequency of diseased patients in all patients with positive test results. The predictive value of a negative test result indicates the frequency of nondiseased patients in all patients with negative test results. The total accuracy of a test indicates the percentage of patients correctly classified by the test.

Sensitivity % = TP/(TP+FN)×100

Specificity % = TN/(FP+TN) ×100

Total accuracy % = (TN+TP)/(TN+TP+FP+FN)×100.

Predictive value of positive test % = TP/(TP+FP)×100

Predictive value of negative test %= TN/(TN+FN)×100

TP, true positives: number of diseased patients correctly classified by the test;
FP, false positives: number of nondiseases patients misclassified by the test;
FN, false negatives: number of diseased patients misclassified by the test;
TN, true negatives: number of nondiseased patients correctly classified by the test.

Receiver operating characteristic (ROC) analysis was used to examine the clinical applicability of 3 biochemical markers for breast cancer. The use of ROC analysis has been suggested as an aid to clinical decision making (Hanley, 1989; Beck, et al., 1986).

The statistical basis for ROC methods having been well described (Swets, 1988; McNeil, et al., 1984; Metz, et al., 1984). Briefly, when a test is used to detect patients having a disease, a critical test value is

usually selected that will best distinguish between the two groups. For tumor markers, results greater than the critical value (test positive) generally denote increased probability of disease. This system defines four groups, those are: test positive with the disease (true positive), test positive without the disease (false positive), test negative with the disease (false negative), and test negative without the disease (true negative). ROC analysis takes advantage of a simplification of these familiar categories. The entire population can be described by just two functions: true-positive fraction (the proportion of test positives among those with the disease) and false-positive fraction (the proportion of test positives among those without the disease). These fractions are linked by any given critical value. For tumor markers such as those discussed in this paper, selection of a higher critical value must result in a smaller false-positive fraction as well as a smaller true-positive fraction. Clearly, selection of a critical value can have a profound influence when tumor markers are compared. The simple expedient of plotting true-positive fraction against false-positive fraction for a range of assay values will overcome many of the difficulties inherent in analysis based on critical value.

The difference between the marker levels in the different groups was calculated with the Mann-Whitney U test. The study for paired pre-operation and post-operation was calculated with the t-paired test.

Chapter 3 Results

3.1 Precision studies

3.1.1 CA 15-3

The IMx® CA 15-3 MEIA kit was used to measure CA 15-3 on IMX. Intra-assay variation was estimated with controls containing low and high values of CA 15-3, the CVs were 2.4 % and 2.3% respectively, the interassay were 5.2% and 7.1% respctively ***(Table3-1)***.

3.1.2 TPA

The IDeal Monoclonal TPA_{cyk} ELASA kit was used to measure TPA. Intra-assay variation was estimated with controls containing low and high values of TPA, the CVs were 3.7 % and 4.4% respectively, the interassay were 3.8% and 2.3% respectively ***(Table 3-2).***

3.1.3 Apolipoprotein(a)

The Mercodia Apo(a) RIA were used to measure Apo(a). Intra-assay variation was estimated with controls containing low and high values of Apo(a), the CVs were 5.7% and 3.1% respectively, the interassay were 5.0% and 2.8% respectively ***(Table 3-3).***

Table 3-1 Intra- and inter-assay variation of CA 15-3 by MEIA on IMx System

	Assay value by supply Mean(range) (U/ml)*	Actual measured mean value (U/ml))	Coefficient of variation		
			Within assay % (n=10)	Between assay % (n=18)	Total assay %
CA 15-3	35 (23.6—43.8)	35.4	2.4	4.6	5.2
	150 (112.5—187.5)	139.9	2.3	7.1	7.5

*IMx CA 15-3 Control (No. 6A75-10)

Table 3-2 Intra- and inter-assay variation of TPA by ELISA on EL340

	Assay value by supply * (ng/ml)	Actual measured mean value (ng/ml)	Coefficient of variation		
			Within assay % (n=15)	Between assay % (n=8)	Total assay %
TPA	1.34 (0.91—1.91)	1.66	3.7	3.8	5.3
	2.91 (2.16—4.00)	3.45	4.4	2.3	5.0

TPAcky Control L and H (Lot No. KA9704).

Table 3-3 Intra- and inter-assay variation of Apo(a) by RIA on Auto-Gamma® 5000 Series Gamma Counting Systems

	Assay value by supply* (U/ml)	**Actual measuredd mean value (U/ml)**	**Coefficient of variation**		
			Within assay % (n=20)	**Between assay % (n=6)**	**Total assay %**
Apo (a)	129 (97—161)	134	5.7	5.0	7.6
	361 (275—458)	376	3.1	2.8	4.2

Mercodia Apo(a) Control L and H (Lot No. 1289), lyophilised Apo(a) concentration(Mean ±3S.D.).

3.2 CA 15-3

3.2.1 CA 15-3 levels in healthy women, patients with benign breast disease and patients with breast cancer.

The distribution of circulating CA 15-3 levels in normal subjects and patients with breast cancer were shown in ***Fig.3-1***. The frequency histogram shows a skewing toward increased CA 15-3 values. It can be seen that patients with breast cancer tend to have higher values of CA 15-3, there is a great deal of overlap between the two groups.

In 97 normal control subjects, the mean and median were 11.1±5.8 U/ml and 9.1 U/ml respectively. CA 15-3 levels were >10 U/ml in 46 subject (47.4%) and >15 U/ml in 22 (22.7%) subjects. Furthermore, only 8 (8.3%) of these subjects had CA 15-3 >20 U/ml and only 3 (3.1%) of these subject had CA 15-3 levels >25 U/ml. In contrast, 188 patients with breast cancer had a mean CA 15-3 level of 19.9±36.1 U/ml and median 13.9 U/ml. 125 of these patients (66.6%) had CA 15-3 levels >10 U/ml, 86 (45.9%) had CA 15-3 levels >15 U/ml, and there were 43 (23.0%) had CA 15-3 levels >20 U/ml and 23 (12.4%) had levels >25 U/ml.

The median value, the 10-90% percentile range of CA 15-3 in groups

of normal controls(median 9.1U/ml), patients with benign breast disease (median 9.8U/ml) and patients with breast cancer (median 13.9U/ml) are shown in ***Figure 3-2.***

These data for patients with breast cancer are significantly different from those normal controls ($p<0.001$), patients with breast cancer had significantly higher CA 15-3 levels (median: 13.9 U/ml) than normal control (median: 9.1 U/ml) ***(Table 3-4, Fig. 3-2)***.

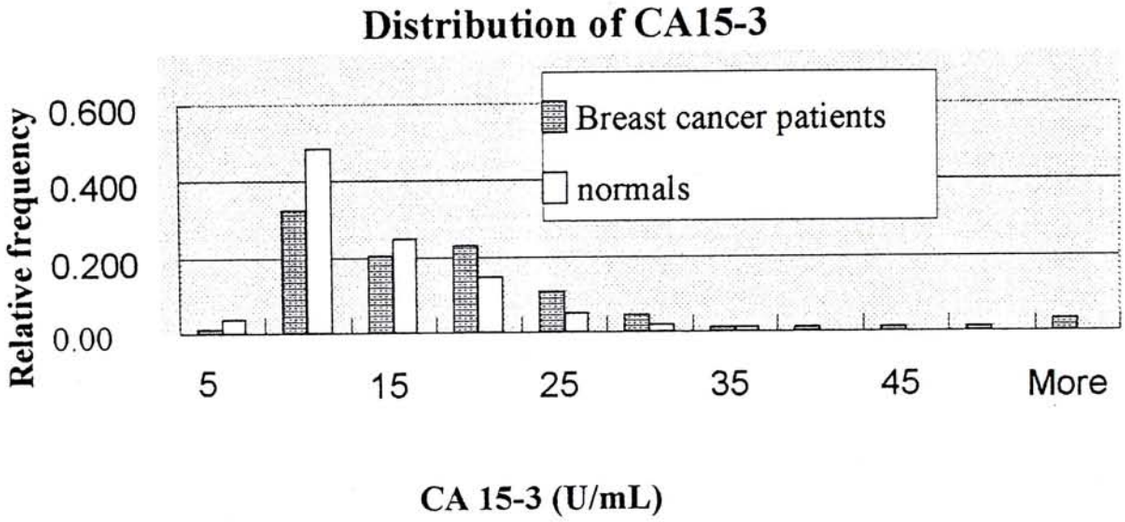

Fig. 3-1 Distribution of the plasma concentration of CA 15-3 in normal controls (n=97) and patients with breast cancer (n=188).

Table 3-4 CA 15-3 results

	Controls	Benign	Breast cancer
N	97	15	188
Mean	11.1	16.8	19.9
Median	9.1	9.8	13.9
SD	5.8	27.4	36.1
Range	3.6-32.5	4.6-114.9	4.6-425.0
P*			0.0013

Mann-Whitney U test to unpaired date (breast cancer vs controls)

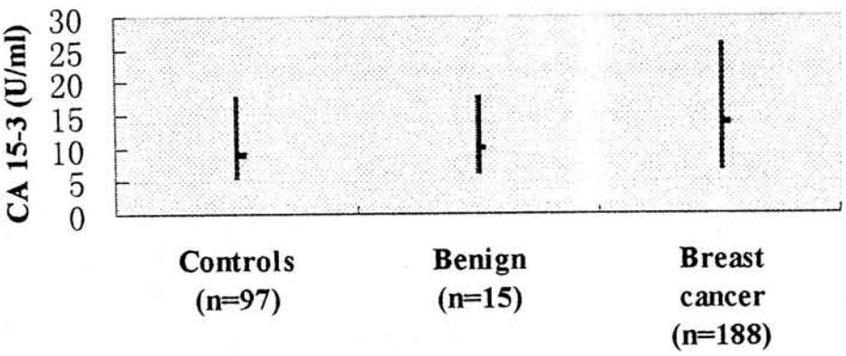

Figure 3-2 Comparison of CA 15-3 plasma levels in the three groups. *(Horizontal bars, median value; thin vertical bar, 10-90th percentile range.)*

3.2.2 Sensitivity, specificity, and total accuracy of preoperative CA 15-3 determination by cutoff value.

The receiver operating characteristic (ROC) analysis of CA 15-3 as a discriminator between normal females and breast cancer patients is shown in ***Fig 3-3***. The curve delineates the relationship between true- and false-positive fraction for a range of critical marker values.

The sensitivity, specificity, and total accuracy were calculated using preoperative tumor markers of patients with breast cancer (n=188), with normal controls (n=97). We differentiated between the cutoff values of 10,15,20,and 22 U/ml. Comparing these results, sensitivity declined from 67% to 18% with an increase cutoff value. Specificity however improved from 53% to 78% and reach to 95%. This make the normal value of 15U/ml we selected and confirmed for health women appear the best choice, because both specificity and total accuracy are above 50%, with a sensitivity of 46%. ***(Table 3-5)***

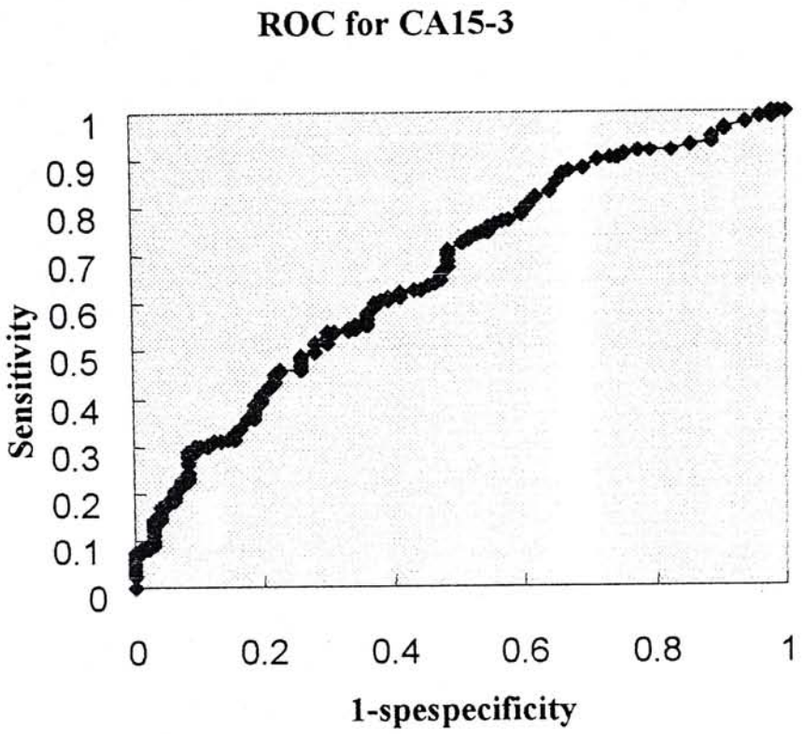

Fig 3-3 Receiver-operator curve for data in CA 15-3. Breast cancer vs normal.

Table 3-5 Sensitivity, specificity, and total accuracy of CA 15-3 determination

CA 15-3 (U/ml) cutoff value	**Sensitivity (N=188)**	**Specificity (N=97)**	**Total accuracy (N=285)**
10	67	53	62
15	**46**	**78**	**57**
20	23	93	47
22	18	95	44

3.3 TPA

3.3.1 TPA levels in healthy women, patients with benign breast disease and patients with breast cancer.

The distribution of circulating TPA levels in normal subjects and patients with breast cancer were shown in ***Fig.3-4.*** The frequency histogram shows a skewing toward increased TPA values. It can be seen that patients with breast cancer tend to have higher values of TPA, there is a great deal of overlap between the two groups.

The distribution of circulating TPA levels in normal subjects and patients with breast cancer were shown in ***Fig.3-4***. In 88 normal control subjects, the mean and median were 0.71±0.54 ng/ml and 0.58 ng/ml respectively. TPA levels were >0.4 ng/ml in 61 subject (69.1%), >0.8 ng/ml in 30 (33.9%) subjects and >1.2 ng/ml in 10 (11.2%) subjects. Furthermore, only 4 (4.4%) of these subjects had TPA >1.4 ng/ml and only 3 (3.3%) of these subject had TPA levels >1.6 ng/ml. In contrast, 115 patients with breast cancer had a mean TPA level of 1.23±3.39 ng/ml and median 0.61 ng/ml. 78 of these patients (67.7%) had TPA levels >0.4 ng/ml, 47 (40.8%) had TPA levels >0.8ng/ml, and there were 28 (24.3%) had TPA levels >1.2ng/ml, 23 (20.0%) had levels >1.4 ng/ml and 19(16.5%) han levels >1.6ng/ml.

The median value, the 10-90% percentile range of TPA in normal controls, patients with benign breast disease and patients with breast cancer are shown in ***Fig. 3-5.***

These data for patients with breast cancer are not significantly different from those normal controls (p=0.053), patients with breast cancer had higher TPA levels (median: 0.61ng/ml) than normal control (median: 0.58ng/ml) ***(Table 3-6, Fig. 3-5)***

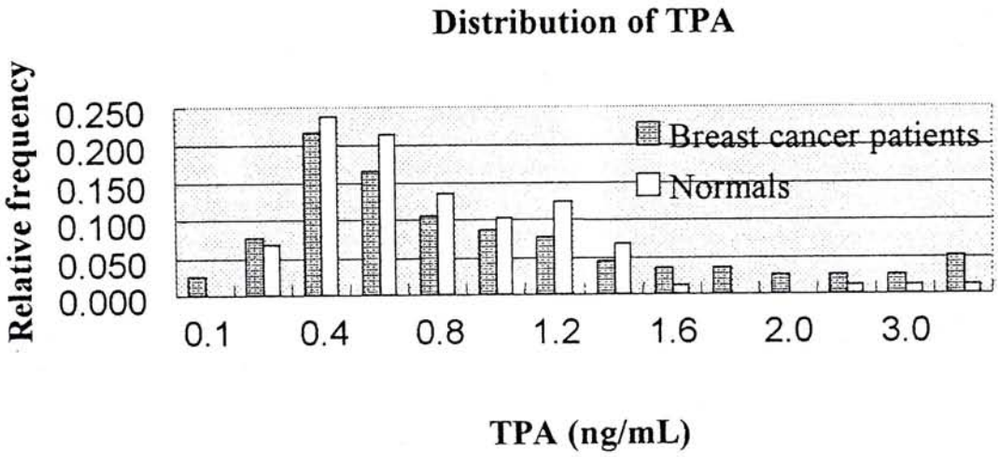

Fig. 3-4 Distribution of the plasma concentration of TPA in normal controls (n=88) and patients with breast cancer (n=115)

Table 3-6 TPA results

	Controls	Benign	Breast cancer
N	88	15	115
Mean	0.71	0.74	1.23
Median	0.58	0.64	0.61
SD	0.54	0.34	3.39
Range	0.13-3.27	0.29-1.26	0.08-35.9
P*			0.0531

** Mann-Whitney U test to unpaired date (breast cancer vs controls)*

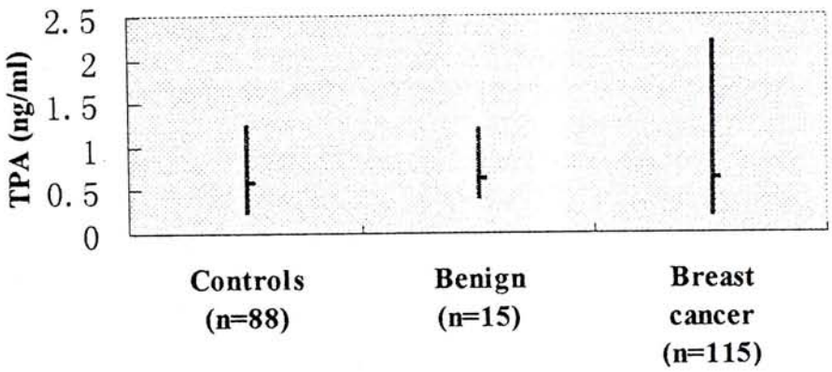

Figure 3-5 Comparison of TPA plasma levels in three groups. *(Horizontal bars, median value; thin vertical bar, 10-90th percentile range).*

3.2.2 Sensitivity, specificity, and total accuracy of preoperative TPA determination by cutoff value.

The relative operating characteristic (ROC) analysis of TPA as a discriminator between normal females and breast cancer patients is shown in ***Fig 3-6***. The curve delineates the relationship between true- and false-positive fraction for a range of critical marker values.

The sensitivity, specificity, and total accuracy were calculated using preoperative tumor markers of patients with breast cancer (n=115), with normal controls (n=88). We differentiated between the cutoff values of 0.50,1.00,1.20,and 1.40 ng/ml (Table 4-4). Comparing these results, sensitivity declined from 59% to20% with an increase cutoff value. Specificity, however improved from 44% to 89% an reach to 95%. This make the normal value of 1.2ng/ml we selected and confirmed for health women appear the best choice, because both specificity and total accuracy are above 50%, with a sensitivity of 25%.

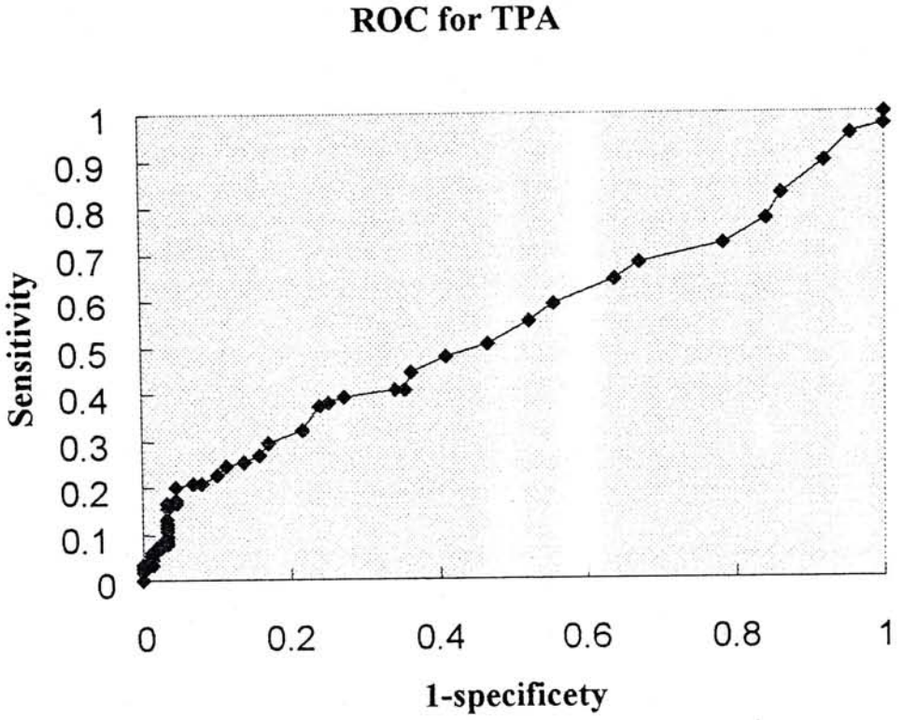

Fig. 3-6 Receiver operator curve for date in TPA. Breast cancer vs normal

Table 3-7 Sensitivity, specificity, and total accuracy of TPA determination

TPA (ng/ml) cutoff value	Sensitivity (n=115) (%)	Specificity (n=88) (%)	Total accuracy (n=203) (%)
0.50	59	44	52
1.00	32	68	48
1.20	**25**	**89**	**53**
1.40	20	95	53

3.4 Apolipoprotein (a)

3.4.1 Apo(a) levels in healthy women, patients with benign breast disease and patients with breast cancer.

The distribution of circulating Apo(a) levels in normal subjects and patients with breast cancer were shown in ***Fig.3-7***. The frequency histogram shows a skewing toward increased Apo(a) values. It can be seen that patients with breast cancer tend to have higher values of Apo(a), there is a great deal of overlap between the two groups.

The distribution of circulating Apo(a) levels in normal subjects and patients with breast cancer were shown in ***Fig. 4-7***. In 97 normal control subjects, the mean and median were 251.4±326.7 U/L and 137.5 U/L respectively. Apo(a) levels were >100U/L in 58 subject (60.5%), >200U/L in 39 (40.9%) subjects and >400U/L in 19 (19.6%) subjects. Furthermore, only 8 (8.2%) of these subjects had Apo(a) >600U/L and only 3 (3.0%) of these subject had Apo(a) levels >800 U/L.In contrast, 188 patients with breast cancer had a mean Apo(a) level of 231.8±220.3 U/L and median 137.2 U/L. 125 of these patients (66.6%) had Apo(a) levels >100 U/L, 75 (40.0%) had Apo(a) levels >200U/L, and there were 37 (19.7%) had Apo(a) levels >400 U/L, 16 (8.5%) had levels >600 U/L and 3(1.6%) had levels >800 u/l.

The median value, 10-90th percentile range of Apolipoprotein(a) in groups of normal controls, patients with benign breast disease and

patients with breast cancer are shown in ***Figure 3-8***

These data for patients with breast cancer are no significantly different from those normal controls (p=0.2978), patients with breast cancer had equal Apo(a) levels (median: 137.2 U/L) with normal control (median: 137.5 U/L) ***(Table 3-8, Fig. 3-8).***

The relative operating characteristic (ROC) analysis of Apo(a) as a discriminator between normal females and breast cancer patients is shown in ***Fig 3-9.*** The curve delineates the relationship between true- and false-positive fraction for a range of critical marker values.

There are no assignment of cut off value if needed because there is no association of Apo(a) to breast cancer.

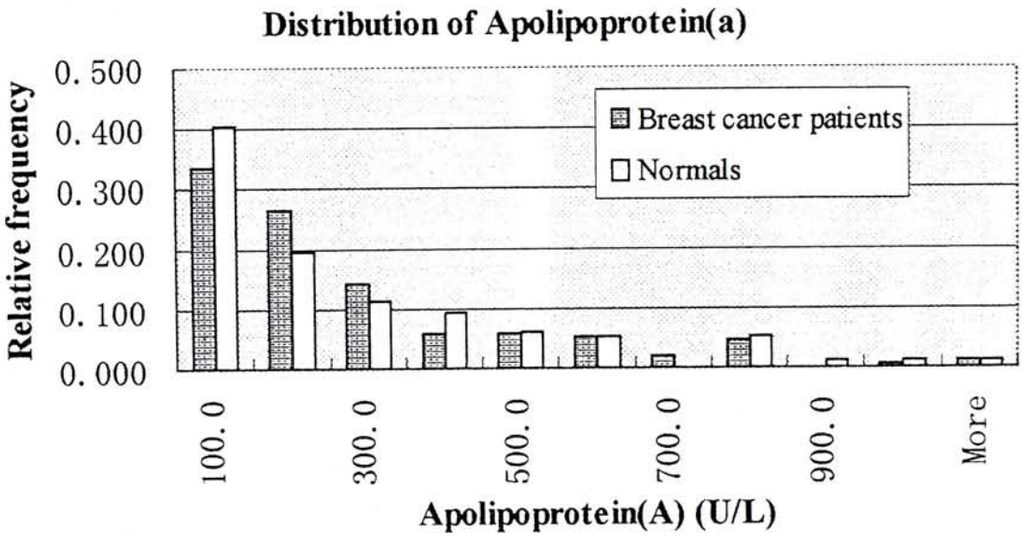

Fig.3-7 Distribution of plasma Apo(a) in normal controls (n=97) and patients with patients with breast cancer(n=188)

Table 3-8 Apo(a) results

	Controls	Benign	Breast cancer
N	97	15	188
Mean	251.4	177.4	231.8
Median	137.5	147.0	137.2
SD	326.7	112.8	220.3
Range	4.8-2635.1	15.6-410.9	1.1-1050.0
P			0.2978

Mann-Whitney U test to unpaired date (breast cancer vs controls)

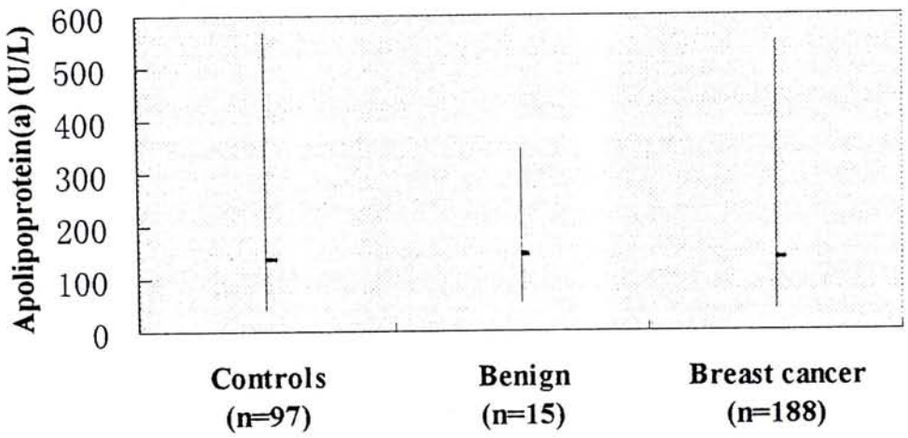

Figure 3-8 Comparison of Apolipoprotein(a) plasma levels in three groups.

(Horizontal bars, median value; thin vertical bar, 10-90th percentile range.)

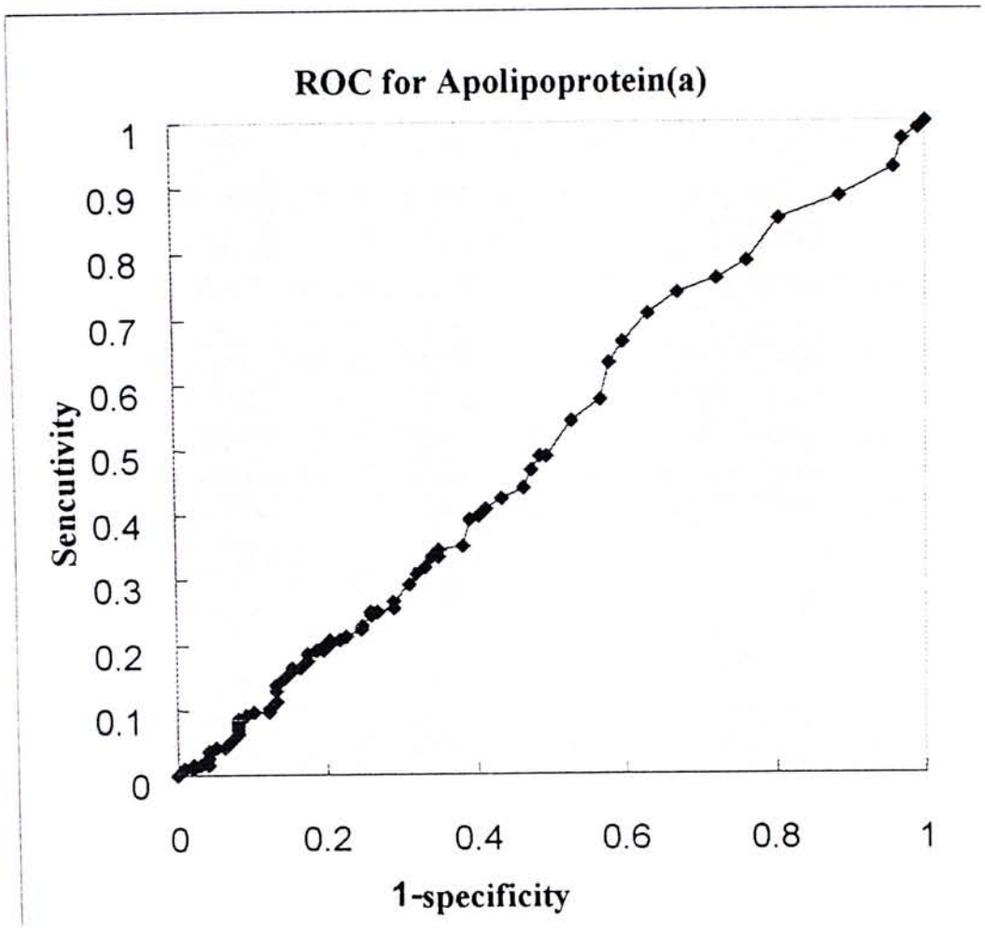

Fig 3-9 Receiver operator curve for Apo(a), Breast cancer vs normal

3.5 Combination test

Disease group comparisons were made using line regression analysis in which all three markers were used as variables to examine the predictive values of using multiple markers as opposed to any single marker. *(Fig 3-10, Fig 3-11, Fig 3-12)*

Combination Testing

Using cutoff 15U/ml for CA 15-3 and 1.2 ng/ml for TPA, values were calculated with date from patients with breast cancer (n=115) and compared with patients with breast disease plus healthy female controls (n=103). Predictive value of positive test = % of patients with positive test results who are diseased.

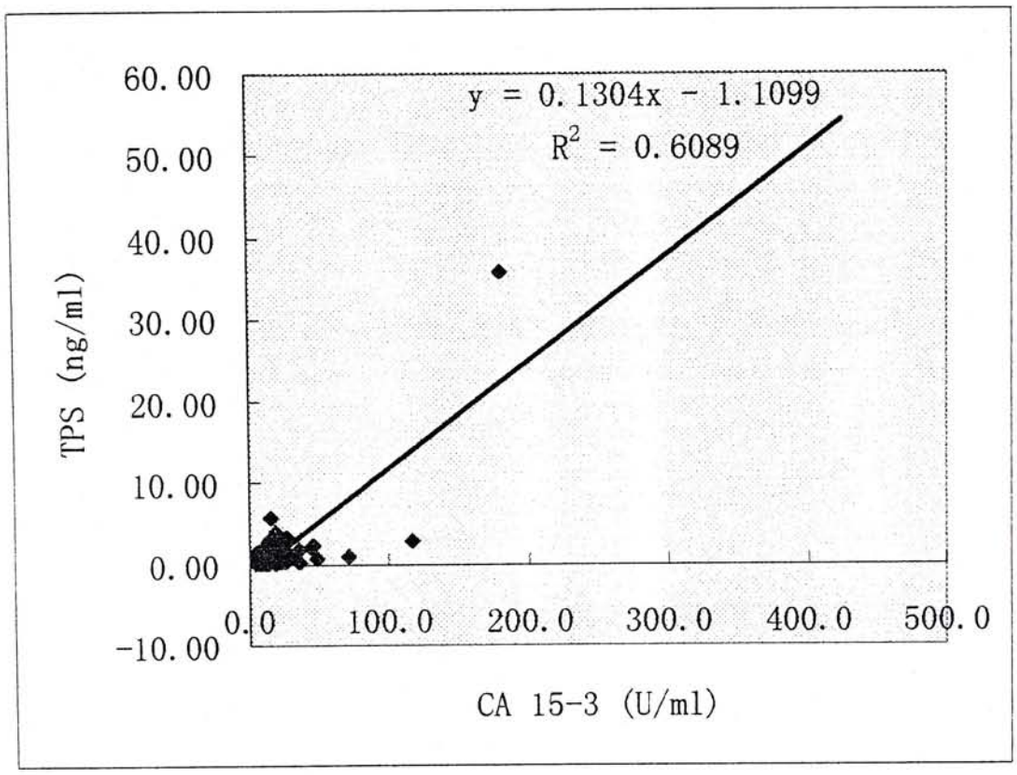

Fig. 3-10. Correlation between TPA and CA 15-3 in pre-operative breast cancer patients (N=115)

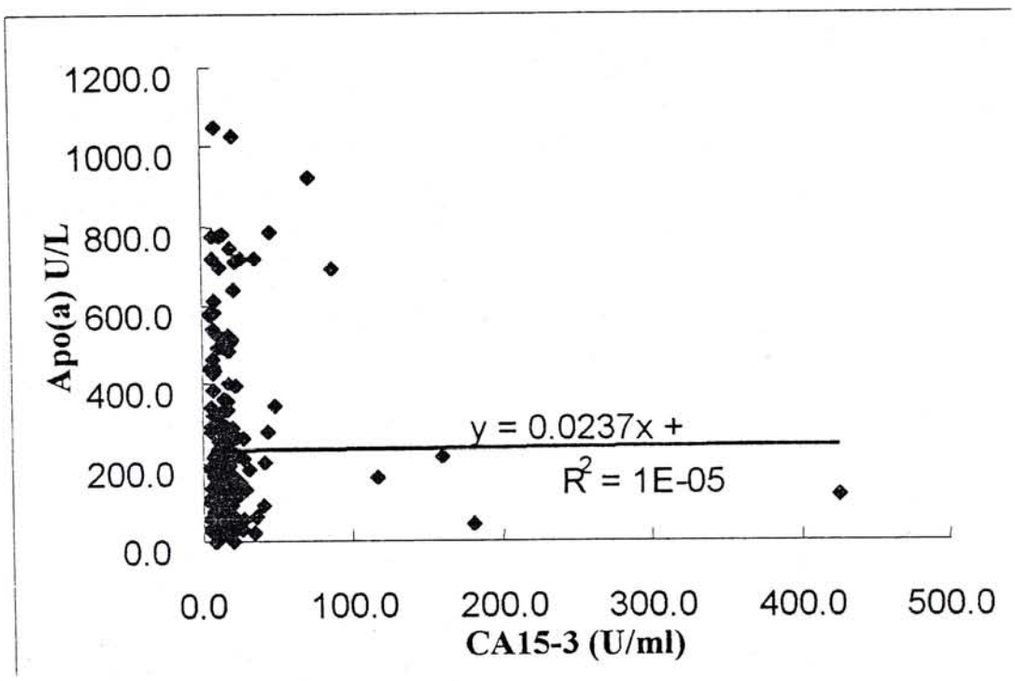

Fig.3-11 Correlation between CA 15-3 and Apo(a) in pre-operative breast cancer patients (n=188)

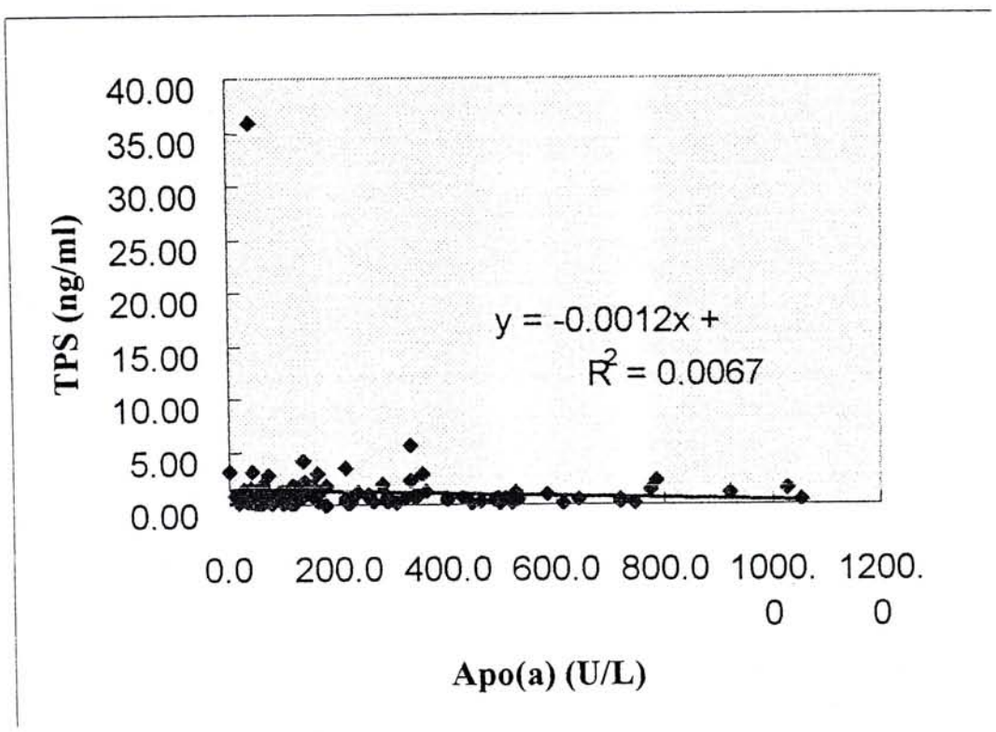

Fig.3-12 Correlation between TPA and Apo(a) in pre-operative breast cancer patients (n=115).

Table 3-9 CA15-3 and TPA in breast cancer

Marker	Sensitivity	Specificity	Positive predictive	Negative predictive
CA-15-3	52(45%)	83(81%)	72%	57%
TPA	28(24%)	92(89%)	72%	51%
CA15-3 and TPA	19(17%)	98(95%)	79%	51%
CA 15-3 or TPA	61(53%)	77(75%)	70%	59%

3.6 Study in pairs

3.6.1 Results of the pairs investigation

The CA 15-3, TPA and Apo(a) plasma levels were measured before and after operation. Pre- and post-operation samples from 60 clinical courses of patients with breast cancer were evaluated with the CA 15-3 assay and Apo(a), 55 patients were evaluated with the TPA assay. The CA 15-3 levels of post-operation (mean±SD, 13.2±6.2) was significant lower than pre-operation (mean±SD, 18.3±18.5) ($p<0.01$). The TPA levels of post-operation (mean±SD, 0.82±0.85) was weakly significant lower than pre-operation (mean±SD, 0.97±1.01) ($p<0.05$). The Apo(a) levels of post-operation (mean±SD, 232.7±196.5) was not significant with the pre-operation (mean±SD, 237.3±209.6) ($p=0.3687$).(***Table 3-7)***

3.6.2 Changes in post-operation compared with the pre-operation levels

The CA 15-3, TPA and Apo(a) levels change in surgery were compared with the pre-operation level. Percent change in these markers were calculated as:

$$\%\ \text{change 15-3} = \frac{\text{CA 15-3}_{post} - \text{CA 15-3}_{pre}}{\text{CA 15-3}_{pre}} \times 100$$

$$\%\ \text{change TPA} = \frac{\text{TPA}_{post} - \text{TPA}_{pre}}{\text{TPA}_{pre}} \times 100$$

$$\% \text{ change Apo(a)} = \frac{\text{Apo(a)}_{post} - \text{Apo(a)}_{pre}}{\text{Apo(a)}_{pre}} \times 100$$

in which marker_{pre} represents the level of pre-operation and marker_{post} represents the level of post-operation.

CA 15-3 levels decreased by at least 10% of the pre-operation level in 40 of 60 patients (66.7%) with surgery. Furthermore, CA 15-3 levels decreased by at least 20% in 27 of these 60 patients after operation. For TPA, there was 30 patients (54.6%) decrease by at least 10% and 21 patients (36.3%) increased by at least 10% after operation. (***Fig. 3-13; Table 3-8)***

Table 3-10 The pre- and post-operation results of CA 15-3, Apo(a) and TPA.

	CA 15-3		Apo (a)		TPA	
	Pre-operation	**Post-operation**	**pre-operation**	**Post-operation**	**Pre-operation**	**Post-operation**
N	60	60	60	60	55	55
Mean	18.3	13.2	237.3	232.7	0.97	0.82
Median	14.3	11.6	158.7	174.3	0.61	0.59
SD	18.5	6.2	209.6	196.5	1.01	0.85
Range	5.5---115.4	5.4---38.0	10.1--1050.2	9.8---930.3	0.17---5.65	0.11-4.20
p*	0.0018		0.3687		0.0412	

* t-paired test

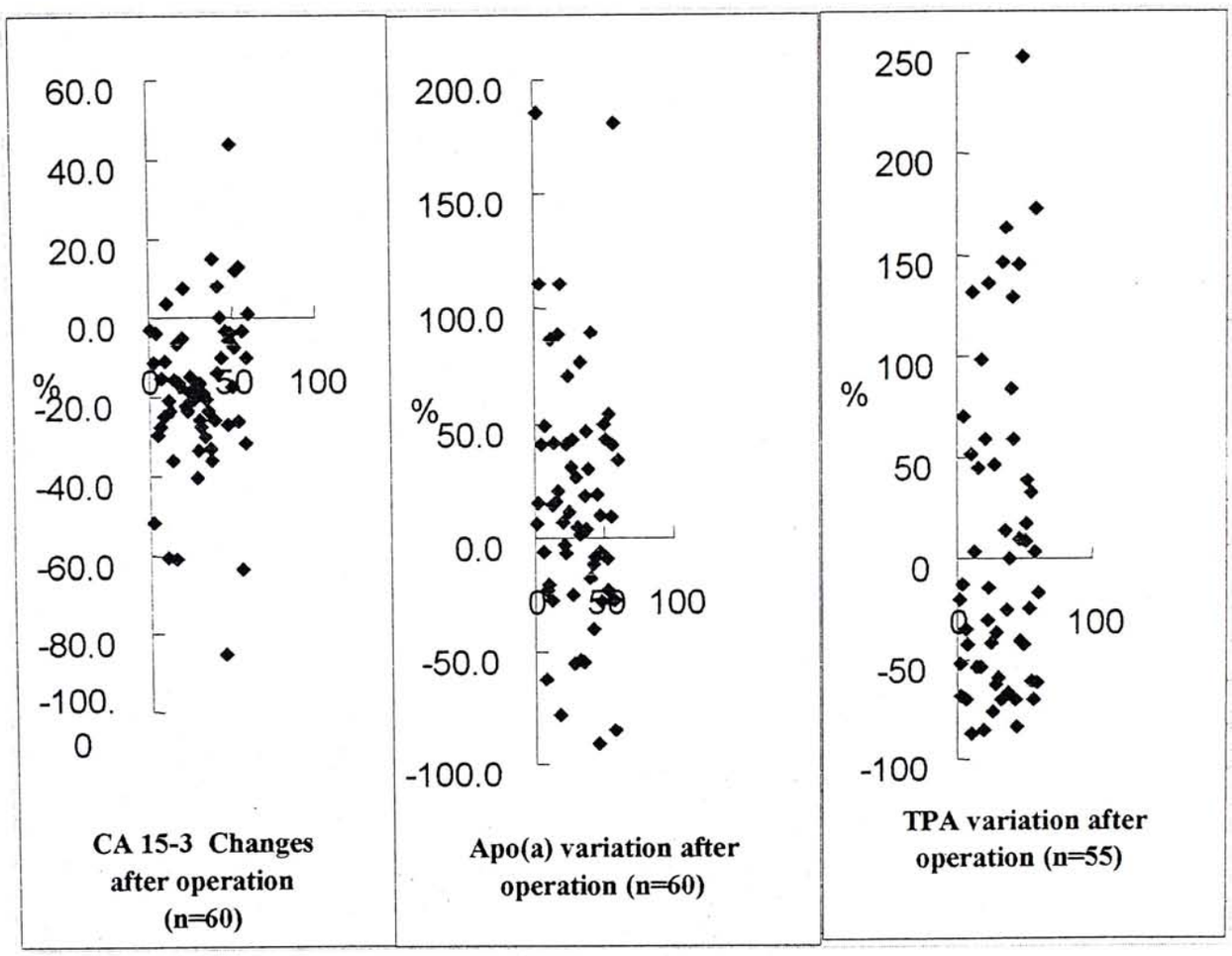

Fig. 3-13 Changes in CA 15-3, TPA and Apo(a) levels after operation.

Table 3-11 The initial changes in the three markers levels after operation.

	Initial changes in markers after operation				
	Decrease		Variation±10%	Increase	
	>20%	>10%		>10%	>20%
CA 15-3 (n=60)	27(45%)	13(21.7%)	16(26.7)	3(5%)	1(1.6%)
TPA (n=55)	27(49.1%)	3(5.5%)	5(9.1%)	2(3.6%)	18(32.7%)
Apo(a)(n=60)	15(25%)	2(3.3%)	13(21.7%)	6(10%)	24(40%)

Chapter 4 Discussion

Breast cancer is the second most cancer and the second leading cause of mortality among women in Hong Kong (Dept. of Health Report, 1993). Early detection will enable prompt treatment and reduce mortality and morbidity. However, in primary breast cancer the 'classic' tumor markers, carcinoembryonic antigen (CEA) (De Jong-Bakker, et al., 1981) and tissue polypeptide antigen (TPA) (Bates et al., 1985), are neither sensitive nor specific enough to indicate the spread of the disease and its clinical course. Monoclonal antibodies (Mab) techniques are more sensitive and specific than previous assays. CA 15-3 uses 115D8 and DF3 (Hayes et al., 1986) and new TPA uses monoclonal antibodies relative to cytokeratins 8 and 18. We examined the relative merits of measuring CA 15-3, TPA and Apolipoprotein(a) levels in the detection and monitoring of breast cancer.

The level used as the upper limit of normal has differed in various reports. Although some variation exits between laboratories, the mean±SD of CA 15-3 level in normal subject ranges from 7 to 18 ± 3-9 U/ml (Colomer et al., 1986; Hayes et al., 1986; Sacks et al., 1987) These data have been used to determine the appropriate cut-off that distinguishes normal subjects from patients with breast cancer. The choice of cut-off level is important for evaluation of the efficacy of the

tumor maker. The situation is complicated by the fact that no standardization of the assays has been made. Different result has been obtained by assays from various manufactures. Some workers (Colomer et al., 1986; Sacks et al., 1987)) used an upper limit of nomal of 40 U/ml while others used 25 U/ml (Pons-Anicet et al., 1987). In our study, the mean±SD of CA 15-3 level in normal subject were 11.1± 5.8, selected cutoff values of 22 U/ml, below which 95% of the normal population will fall. At this cutoff, the sensitivity for breast cancer was 18%. The cut-off values to use are controversial since sensitivity and specificity depend the cut-off. There were study plotted receiver operating characteristics (ROC) curves for CA 15-3(Eskelinen et al., 1989; Hayes et al., 1986; Pons-Anicet et al., 1987). In this study, using ROC, selected cutoff at 15 U/ml, the sensitivity and specificity were 46% and 78% respectively.

In one study , the cut-off level (95% specificity) was 30U/ml for CA 15-3, the diagnostic sensitivity was 32% for the detection of breast cancer.(Giai et al, 1996) CA 15-3 basal levels were solely related to tumor size and nodal involvement. The CA 15-3 levels appear to be independent of histology estrogen receptor content, or histologic grade of the primary tumors, but are related to the stage and extent of disease (Hayes et al., 1986; Colomer et al., 1989; Safi et al., 1989). There were study indicated that CA 15-3 were insufficiently sensitive and specific to be used to screen the general population (Tondini, et al., 1989).

In this study, The IDeal Monoclonal TPA_{cyk} ELASA is a test for the determination the key epitopes of TPA (Tissue Polypeptide Antigen)

related to cytokeratins 8 and 18, the monoclonal antibody appeared to detect more specific epitope. In our study, at the cutoff 1.4 ng/ml, the sensitivity and specificity were 20% and 95% respectively. Using ROC analysis, selected cutoff 1.2ng/ml, the sensitivity and specificity were 25% and 89% respectively. Using cutoff 15 U/ml for CA 15-3 and 1.2 ng/ml for TPA, the false-positive rates for CA 15-3 and TPA in normal subject were 19% and 11%, respectively. Another study shown that the false -positive rate were 16% and 4%.(Barak et al.,1990). In one study, the cut-off levels (90% specificity) the diagnosis sensitivity of the TPA test was 23% and 13% for the CA 15-3 test. When the cut-off were determined at 95th percentile level, the diagnostic sensitivity of the TPA and CA 15-3 were 12% and 6% respectively (Eskelinen et al, 1994).

The mean and median levels of CA 15-3 and TPA in patients with breast cancer were significantly higher than those of normal controls. Our study demonstrated that CA 15-3 was more sensitive than TPA. TPA, has been regard as a risk factor rather than a real tumor marker (Nicolini er al., 1989), However, in our study TPA at selected cut off levels showed a good specificity. Correlation was not found between TPA and CA 15-3, so the use of CA 15-3 together with TPA increases the sensitivity. The combined sensitivity of CA 15-3 and TPA increased to 53%, but the main problem with CA 15-3/ TPA stems from their low combined specificity (also found by Eskelinen et al., 1989) of 75%, while their individual specificity was 83% for CA 15-3 and 92% for TPA.

In our study indicates that CA 15-3 and TPA had limited value in

breast cancer diagnosis, the combined use of tumor markers in breast cancer diagnosis may have some clinical value, so far such test have too low a specificity to be of practical value in screening.

Human mammary tissue metabolizes lipids from plasma, a process affected by female gonadal hormones. Both benign and malignant proliferation of breast tissue in women has associated with changes in plasma lipid and lipoprotein levels. The ratio of apolipoprotein A-I to serum Apolipoprotein B levels at time of biopsy has been referred to be the best predictor of cancer recurrence.(Lane DM et al, 1995). Breast cancer was proved to be associated with increased lipoprotein(a) (Alexopoulos et al, 1987).

In our study, the plasma levels of Apolipoprotein (a) in the breast cancer patients group not differed significantly from normal controls ($p=0.2978$). The variation of Apo(a) in our study could be attributed to other causes, such as smoking, alcohol consumption, atherosclerotic vascular disease, hypertension, diabetes mellitus, or hyperlipidemia.

The clinical application of circulating tumor markers remains a controversial subject in terms of useful methods and correct interpretation of findings. In particular and despite numerous investigations in the field, we do not have highly specific and sensitive biological markers in breast cancer. Nevertheless, many researchers often utilize circulating tumor markers in various phases of this malignancy to obtain additional information about disease extent and clinical course.

Chapter 5 Conclusions

1. The is an association between CA 15-3 and occurrence of breast cancer.
2. There is an association between new TPA and occurrence of breast cancer.
3. Apolipoprotein(a) is not associated with breast cancer.
4. CA 15-3 and TPA in combination improve specificity.
5. CA 15-3 and/or TPA are not useful for screening, in conjunction with other diagnostic tests, both markers may be useful for diagnosis & monitoring of breast cancer.

References

Abe M, and DW Kufe (1987) Identification of a family of high molecular weight tumor-associated glycoproteins J. Immunol.139, 257-261.

Abelev, GI, Perova SD, Khramkova NI, et al.(1963) Production of embryonal α-globulins by trasplaintable mouse hepatomas. Trasplantation, 1:174-180.

Albers, J. J., S. M. Marconina and M. S. Lodge. (1990). The unique lipoprotein (a). Properties and immunochemical measurement. Clin. Chem. 36, 2019-2026

Alexopoulos CG, Blatsios B, Avgerinos A. (1987) Serum lipids and ;lipoprotein disorders in cancer patients.Cancer. 60(12):3065-3070.

Bahr,J.,Carlsson,K. & Luning,B(1988). An epitope in Coil 2B of cytokeratin 8. Acta Chem.Scand., B42,442-447

Barak M, Steiner M, Finkel B, Abrahamson J, Antal S, Gruener N.(1990) Ca-15.3, TPA and MCA as Markers for Breast Cancer. Eur J Cancer,5:577-580

Barbareschi M, Leonardi E, Mauri FA, et al.(1992) P53 and c-erbB-2 protein expression in breast carcinomas: An immunohistochemical study including correlation with receptor status, proliferation markers, and clinical stage in human breast cancer. Am J Clin Pathol 98:408-418.

Barclay, M. and V. P. Skipski (1975). Lipoproteins in relation to cancer. Prog. Biochem. Pharmacol. 10,75-111.

Bates SE, Longo DL (1985) Tumor markers: value and limitations in the management of cancer patients. Cancer Treat Rev,12:163-207.

Baum M.(1982) Breast cancer :the fact . New York: Oxford University Press.1982.

Beard DB, Haskell CM (1986) Carcinoembryonic antigen in breast cancer .Am J Med 80:241-245

Beck JR and Shultz, E. K. (1986) The use of relative operating characteristic(ROC)

curves in test performance evaluation. Arch. Pathol. Lab. Med.,110:13-20.

Bence-Jones H (1867) Papers on chemical pathology. Lecture III. Lance, ii:269-272.

Björklund,B. & Björklund,V.(1957) Antigenicity of pooled human malignant and normal tissues by cyto-immunological technique: Presence of an insoluble heat-labile tumor antigen.Int.Allergy,10,153-184

Bjorklund,B., B.Wiklund, B.Luning, K. Andersson, E. Kallin, and V. Bjorklund (1980), Tumor Diagnostik 2,78-84

Bodenmuller, H., Donie, F., Kaufmann, M. & Banauch, D. (1994) The tumor markers TPA, TPS, TPAcyt AND cyfra 21-1 react differently with the keratins 8,18 and 19. Int. J. Biol. Mark.

Bombardieri E, Gion M.(1992) Mucin-like cancer associated antigen (MCA) as available circulating tumor marker for breast cancer. In: Serological Cancer Markers. S Sell, Ed. Totowa, NJ, The Humana Press, 1992, pp 341-354.

Bon GG, Kenemans P, Yedemac A, et al.(1990) Clinical Relevance of the Tumor Marker CA 15-3 in the Management of Cancer Patients. In From Clone To Clinic. The Netherlands; Kluwer Academic Publishers,111-22.

Bray CR, Koda JE, Gaur PK.(1987) Serum levels and biochemical characteristics of cancer associated antigen CA-549, a circulating breast cancer marker. Cancer Res 47:5853-60.

Browning CK, McFarlane NP, Horobin TM, et al.(1988) Evaluation of the comparative clinical utility of CA 15-3 and mucinous carcinoma-associated antigen(MCA) in the management of breast carcinoma. Ann Clin Biochem 25(Suppl)545-65

Capony F, Garcia M, Veith F, et al. (1982) Antibodies to the estrogen induced 52k protein released by human breast cancer cells. Biochem Biophys Res Commun 108: 8-15.

Capony F, Rougeot C, Montcourrier P, et al. (1989) Increased secretion, altered processing and glycosylation of pro-Cathepsin D in human mammary cancer cells. Cancer Res 49: 3904-3909.

Cattoretti G, Rilke F, Andreola S, et al. (1988). P53 expression in breast cancer. Int J Cancer 41: 178-183.

Chan, DW, Beveridge RA, Bruzek D. (1988) Monitoring breast cancer with CA 549. Clin. Chem., 34:2000-2004.

Cheung SY (1992) Breast cancer in Hong Kong - the need for early detection. J Hong Kong Med Assoc 4:248-252.

Chu TM. (1992) Prostate specific antigen. In: Serolocical Cancer Markers. S. Sell, Ed. Totowa, NJ, The Humana Press, 1992 pp. 99-115.

Colomer R, Sole LA, Mavarro M et al (1986) CA 15-3 early results of a new breast cancer marker. Anticancer Res 6:683-4.

Colomer[1] R, Ruibal A, Genolia G, et al.(1989) Circulating CA 15-3 Levels in the Postsurgical Follow-up of Breast Cancer Patients and in Non-malignant Diseases. Breast Cancer Res Treat 13:123-33

Colomer[2] R, Ruibal A, Salvador L.(1989) Circulating Tumor Marker Levels in Advanced Breast Carcinoma Correlate with the Extent of Metastatic Disease Cancer 64:1674-1681

C P Pang (1995) Tumor markers. Study Guide:2-6

De Jong-Bakker M, Hart AAM, Persijn JP, Cleton FJ, Cleton FJ. (1981) Prognostic significance of CEA in breast cancer: a statistical study. Eur J Cancer, 17:1307-1313.

Dnistrian A, Schwartz M, Greenberg E, et al.(1991) CA 15-3 and carcinoembryogenic antigen in the clinical evaluation of breast cancer.Clinica Chimica Acta 200:81-94

Early breast cancer trialists' collaborative group T(1992).Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy: 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Lancet 339:1-15,71-85.

Eddy DM, Hasselblad V, McGivney W, Hendee W(1988) The valre of mammography screening in women under age 50 years. JAMA 259:1512-1519.

Eddy DM: Screening for breast cancer(1989) Intern Med 111:389-399.

Eskelinen M, Tikanoja S, Collan Y.(1989)Use of tumor markers CA-15.3,MCA and CEA in breast cancer diagnostics .F Tumor Marker Oncol 34,39-44.

Eskelinen M, Hippelainen M, Kettumen J, Salmela E, Salmela E, et al.(1994) Clinical value of serum tumour markers TPA,TPS,TAG12,CA 15-3 and MCA in breast cancer diagnosis; results from a prospective study. Anticancer Res. 14:2B, 699-703.

Finlay CA, Hinds PW, Levine AJ. (1989). The p53 proto-oncogene can act as a

suppressor of transformation. Cell 57: 1083-1093.

Fletcher SW, Fletcher RH(1992). The breast is close to the heart. Ann Intern Med 117:969-971.

Gail MH (1979). Some statistical methods for evaluating immunodiagnostic tests. In: Herverman R, McIntire R, eds. Immunodiagnosis of cancer. New York: Marcel Dekker, 1979:20-37.

Giai M, Roagna R, Ponzone R, Biglia N, Sgro L, Perona M, Sismondi P. (1996) TPS and CA 15-3 serum values as a guide for treating and monitoring breast cancer patients. Anticancer Res. 16(2):875-881.

Gion[1] M, ,Mione R, Gatti C, Dittadi R, Leon A, Nascimben O, Pizzorno B, Bruscagnin G (1990) Is tissue polypeptide antigen still a useful tumor marker in breast carcinoma? Comparison with CA15-3 and MCA. Tumori 76:360-364

Gion[2], M., Mione, R., Gatti, G., Dittadi, R., Leon, A. E., Nascimben, O., Pizzorno, B. & Bruscagnin, G. (1990) Tissue polypeptide antigen in tumor cytosol: A new prognostic indicator in primary 1breast cancer. Breast Cancer Res. Treat. 17, 15-21.

Gion M, Mione R, Nascimbe O,et al.(1991) The tumor associated antigen CA 15-3 in primary breast cancer. Evaluation of 667 cases. Br J Cancer 63:809-813

Gion, M., Mione, R., Pappagallo, G. L., Gatti, C., Nascimben, O., Sampognaro, E. & Meo, S. (1994). Biochemical parameters for prognostic evaluation in patients with breast cancer. Anticancer Research 14, 693-698.

Gion, M., Mione, R., Pappagallo, G. L., Nascimben,O., Brandes, A., Vinante, O. & Bruscagini, G. (1993) Tissue polypeptide antigen in breast cancer cytosol. A new effective prognostic indicator. Eur. J. Cancer 29A, 66-69.

Gion, R. Mione, P.Barioli, P.Sartorello and G.Capitanio(1994) Tissue Polypeptide Antigen and Tissue Polypeptide Specific Antigen in Primary Breast Cancer ,Evaluation in Serum and Tumour Tissue Eur. J.Clin.Ghem.Clin.Biochem.Vol.32,,pp.779-787

Gold P, Freeman SO (1965) Demonstration of tumor specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J Exp Med 121:439-462.

Greene GL, Sobel NB, King WJ et al.(1984) Immunochemical studies of estrogen receptors. J Steroid Biochem 20:51-56

Green S, Gronemeyer H, Chambon P (1987) Structure and function of steroid

hormone receptors, in Sluyser M (ed): Growth factors and oncogenes in breast cancer, England, Ellis Horwood,1987,pp7-28.

Grigioni WF, Garbisa S, D'Errico A, et al, (1991) Evaluation of hepatocellular carcinoma aggressiveness by a panel of extracellular matrix antigens. Am J Pathol 138:647-654.

Gullick WJ (1990) The role of the epidermal growth factor receptor and the c-erbB-2 protein in breast cancer. Int J Cancer 5: 55-61.

Hageman PH, Tweel van den J, Hilgers J et al.(1984) Sweat glands and salivary glands as model system for the characterization of monoclonal antibodies against differentiation antigens of the human mammary gland. Protides of Biological Fluids 31:1009-1012.

Hanley, J. A. (1989) The place of statistical methods in radiology(and in the bigger picture). Invest. Radiol.,24:10-16 1989.

Hayes DF, Sekine H, Ohno T et al. (1985) Use of murine monoclonal antibody for detection of circulating plasma DF 3 antigen levels in breast cancer patients .J Clin Invest 1985 :75:1671-1677.

Hayes DF, Zurawski V, Kufe D.(1986) Comparison of circulation CA 15-3 and Carcinoembryogenic Antigen levels in Patients with breast cancer. J Clin Onc 4:1542-50.

Hayes DF, Tondini C, Kufe DW.(1992) Clinical applications of CA15-3. In: Serological cancer markers. S Sell, Ed. Totowa, NJ, The Humana Press, 1992 pp.281-307.

Henderson IC, Garber JE, Breitmeyer JB,et al (1990). Comprehensive management of disseminated breast cancer. Cancer 66:1439-1448.

Hikens J, Kroezen V, Bonfrer JMG, Brunning PF, Hilgers J, Eijkeren van M.(1985) a sandwich-radioimmunoassay for a new antigen (MAM-6) present in the sera of patient with metastasized carcinomas. Protides of Biological Fluids 2:651-653.

Hikens J, Kroezen V, Bonfrer JM,et al.(1986) MAM-6 Antigen, a New Marker for Breast Cancer Monitoring ,Cancer Res 46: 2582-7.

Hilkens[1] J, Buijs F, Hilgers J, et al.(1984) Monoclonal antibodies against human milk-fat globule membranes detecting differentiation antigens of the mammary gland and its tumors. Int J Cancer 34:197-206.

Hilkens[2] J, Hilgers J, Buijs F, et al. (1984) Monoclonal antibodies against human milk-fat globule membranes useful in carcinoma research. Prot Biol Fluids

31:1013-6.

Hong Kong Department of Health.(1993) Main causes of death from cancer in Hong Kong :1982 to 1992. Annual Report 1991/1992. Hong Kong: Department of Health, 1993.

Kamboh Ml et al.(1991) Expressed hypervariable polymorphism of apolipoprotein(a). Am Jhum Genet 1991:49:1063-1074

Kiang DT, Greenberg LJ(1990) Tumor marker kinetics in the monitoring of breast cancer. Cancer 65:193-199

Kostner,G,M.(1983).Apolipoproteins and lipoproteins of human plasma: significance in health and disease. Adv.Lipid Res. 29,1-299.

Kufe D, Inghirami G, Abe M, et al.(1984) Differential reactivity of a novel monoclonal antibody(DF3) with human malignant vs benign breast tumors. Hybridoma 3:223-32.

Kufe DW, Inghirami G, Abe M, Hayes DF, Justi-Wheeler H, Schlom J. 1984 Differential reactivity of a novel monoclonal antibody (DF 3) with human malignant versus breast tumors. Hybridoma 1984;3:223-232.

Lackner C et al.(1991) Malecular basis of apolipoprotein(a) isoform size heterogenity as revealed by pulsed field electrophoresis. J Clin Invest 1991;87:2153-2161

Laurence DJR, Stevens U, Bettelheim R et al, (1972). Role of plasma carcinoembryonic antigen in diagnosis of gastrointestinal, mammary, and bronchial. Br J Med 1972:605-609.

Lazarides, E.(1980) Intermediate filaments as mechanical integrators of cellular space. Nature, 282,249-256

Lazarides,E.(1982) Intermediate filaments:a chemically heterogenous developmentally regulated class of proteins.Ann Rev.Biochem.,51,219-250

Leube,R.E., Bosch,F.X., Romano,W., Zimbelman,R., Hofler, H. & Franke W.W.(1986) Cytokertin expression in simple epithelia.III Detection of mRNAs encoding human cytokeratins nos. 8 and 18 in normal and tumor cells by hydridization with cDNA sequences in vitro and in situ.Differentiation, 33,69-85

Levine AJ, Momand J, Finlay C, at al, (1991). The p53 tumor suppressor gene. Nature 351: 453-455.

Lundi J, Thor A, Marnza R et al.(1985) Monoclonal antibody DF3 correlates with

tumor differentiation and hormone receptor status in breast cancer patients. Breast Cancer Res Treat 5:269-276.

Luning,B.&Nilsson, U. (1983) Sequence homology between TPA and intermediate filament proteins.Acta Chem.Scane.,B37,731-735

MBewu AD and Durrington PN.(1990) Lipoprotein(a): Structure, properties and possible involvement in trombogenesis and atherogenesis. Atherosclerosis 1990;85:1-14.

McNeil, B.J., and Hanley, J. (1984) Statistical approaches to the analysis of receiver operating characteristic(ROC) curves. Med. Decis. Making, 4:137-150.

Metz, C.E., Wang, P-L., and Kronan, H. B. (1984) A new approach for testing the significance of differences between ROC curves from correlated data. In: F. Deconink (ed.), Information Processing in Medical Imaging, pp. 432-445. The Hague, The Netherlands: Nijhoff,1984.

Meden H, Neues S, Roben-Kampken S, et al.(1995) A clinical, mammographic, sonographic, and histologic evaluation of breast cancer. Int J Gynecol Obstet 48:193-199

Mellerick,D.M., Osborn,M.&Weber,K.(1990) On the nature of serological TPA;monoclonal keratin 8,18 and 19 antibodies react differently with TPA prepared from human cultured cells and TPA in human serum.Oncogene,5,1007-1017

Moll, R., Schiller,D.L. & Frake,W.W.(1990) Identification of protein IT of the intestinal cytoskelton as a novel type I cytokeratin with unusual properties and expression patterns.J.Cell Biol.,111,567-580

Moll, R., Franke,W.W. & Schiller, D.L.(1982) The catalog of human cytokeratins:Patterns of expression in normal epithelia,tumors and cutured cells.Cell, 31,11-24

Mountford, C. E., G. L. May, L. C. Wright, W. B. Mackinnon, M. Dyne, K. T. Holmes, C. Van Haaften-Day and M. H. N. Tattersall (1987). Proteolipid in the plasma of a patient with a borderline ovarian tumor identified by magnetic resonance spectroscopy: a case history. Lancet 1 (8537), 829-834.

Niendorf, A., A. Stang, U. Beisiegel, A. Peters, H. Nagele, A. Gebhardt and R. Kuse (1992). Elevated lipoprotein(a) levels in patients with acute myeloblastic leukaemia decrease after successful chemotherapeutic treatment. Clin. Investig. (Germany) (70)8,683-685.

Nicolini A, Capri A, Di Marco G, Giuliani L, Giordani R, Palla S.(1989) A rational

postoperative follow-up with CEA TPA and urinary hydroxyproline in breast cancer patients. Cancer 1989, 63:2037-2046.

Nyström L, Rutqvist LE, Waall S , et al.,(1993). Breast cancer screening with mammography: Overview of Swedish randomised trials. Lancet 341:973-978.

Ochuchi N, Nose M, Abe R et al. (1984) Lectin-binding patterns of breast carcinoma: Significance of structural atypism. Tohoku J Exp Med 143:491-499.

Oehr, P., Q.Liu, M.Nap, B.Schultes, M.lechmann, Y.Ota, and H.Y.Jin (1991),Tumordiagn. Ther.submitted.

Oesterling JE. (1991) Prostate specific antigen: A critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J Urol, 145: 907-923.

Ostrowski JL, Sawan A, Henry L, et al, (1991) p53 expression in human breast cancer related to survival and prognostic factors: An immunohistochemical study. J Pathol 164:75-81.

Polly S.Y.Cheung.(1992) Breast cancer in Hong Kong –the need for early detection J Hog Kong Med Assoc Vol 44,No.4,December 1992

Pons-Anicet DM, Krebs BP, Mira R, Namer M,(1987) Value of CA-15.3 in the follow-up of breast cancer patients. Br F Cancer 55,567-569

Prosser J, Thompson P, et al, (1990) Evidence that p53 behaves as a tumor suppressor gene in sporadic breast tumors. Oncogene 5:1573-1578.

Quinlan,.A.,Schiller,D.L.,Hatzfeld,M.,Achtstatter,T.,Moll,R.,Jorcano,J.L.,Magin,T. M.&Franke,W.W. (1985) Patterns of expression and organization of cytokeratin intermediate filaments. In: E.Wang, D.Fishman,R.R.Hiem & T.T.Sun(eds.) Intermediate elaments,Ann.N.Y.Acad.Sci.,455,282-306

Redelius,P., Luning,B. & Bjorklund, B.(1980) Chemical studies of tissue polypeptide antigen(TPA).II Partial amino acid sequence of cyanogen bromide fragments of TPA subunit B1.Acta Chem. Scand.,B34,265-273

Robertson JFR, Pearson D, Price MR, et al.(1988) Assessment of Four Monoclonal Antibodies as Serum Markers in Breast Cancer , Eur J Cancer 26:1127-1132

Sacks MPM, Stacker SA, Thomson CH et al (1987) Comparison of mammary serum antigen (MSA) and CA 15.3 levels in the serum of patients with breast cancer. Br J Cancer 56:820-4.

Safi F, Kohler I, Röttinger E, Suhr P, Beger HG (19890 Int J Biol Markers 4:207-214.

Safi F, Kohler I, Röttinger E, beger ,H-G.(1991) The Value of the Tumor Marker CA 15-3 in Diagnosing and Monitoring Breast Cancer. Cancer 68:574-582

Scanu, A. M. and L. Scandiani (1991). Lipoprotein (a): Structure, biology and clinical relevance. Adv. Intern. Med. 36, 249-270

Schwartz MK. (1992) The role of the laboratory in the prevention and detection of chromic disease. Clin Chem 38: 1539

Sekine H, Ohno T, Kufe DW .(1985) Purification and characterization of a high molecular weight glycoprotein detectable in human milk and breast carcinomas. J Immunol 1985;135:3610-3615.

Shapiro S, Venel W, Straz I, et al .(1985) Selection, follow-up and analysis in the Health Insurance Plan Study: A randomized trial with breast cancer screening . Natl Cancer Inst Monogr 67:65

Siddiqui J, Abe M, Hayea DF, Shani E, Yunic E, Kufe DW .(1988) Isolation and sequencing on and cDNA coding for the human DF 3 breast carcinoma-associated antigen .Proc Natl Acad Sci USA 1988:85:2320-2323.

Silver H, Archibald B-L, Ragaz J, Coldman A.(1991) Relative Operating Characteristic Analysis and Group Modeling for Tumor Markers: Comparison of CA 15-3, Carcinoembryogenic Antigen, and Mucin-like Carcinoma-associated Antigen in Breast Carcinoma. Cancer Research 51:1904-1909

Skipski, V. P., Barclay, F. M. Archibald and C. C. Stock (1975). Tumour proteolipids. Proc. Biochem. Pharmacol 10, 112-134

Spyratos F, Maudelonde T, Brouilled JP, et al. (1989) Cathepsin D: An independent prognostic factor for metastasis of breast cancer. Lancet 8672: 1115-1118.

Stacker SA, Thompson CH, Riglar C, McKenzie I.(1985) A new breast carcinoma antigen defined by a monoclonal antibody. J Nat Cancer Inst 75:801-4.

Stamon DJ, Godolphin W, Jones LA, et al, (1989). Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 254: 1153-1160.

Steinart,P.M., Steven,A.C. & Roop,D.R.(19850 The molecular biology of intermediate filaments.Cell,42,411-419

Sundstrom,B. & Stigbrand,T.(1990)Two-site enzyme linked immunosorbent assay for cytokeratin 8Int.J.Cancer,46,604-607

Swets, J. A. (1988) Measuring the accuracy of diagnostic systems.

Scitnce(Washington DC), 240:1285-1292.

Tandon AK, Clark GM, Chamness GC,et al (1990) Cathepsin D and prognoses in breast cancer. N Engl J Med 322:297-302

Taketa K (1992) Alpha-fetoprotein in the 1990s. In: Serolocical Cancer Markers. S. Sell, Ed. Totowa, NJ, The Humana Press, 1992 pp. 31-46.

Taylor-Papadimitriou J ,Gendler S.(1988) Molecular aspects of mucins. Cancer Rev.11-12:11-24.

Thorpe SM, Rochefort H, Garcia M, et al. (1989) Association between high concentration of M 52,000 cathepsin D and poor prognosis in primary human breast cancer. Cancer Res 49: 6008-6014.

Tobias R, Rothwell C, Wagner J, et al.(1985) Development and Evaluation of a radioimmunoassay for the detection of a monoclonal antibody defined breast tumor associated antigen 115D8/DF3.Clin Chem 31:986

Toyes DF, Zurawski V, Kufe D.(1986) Comparison of circulating CA15-3 and carcinoembryogenic antigen levels in patients with breast cancer. J Clin Onc 4:1542-50.

Tondini C, Hayes DF, Gelman R, et al.(1988) Comparison of CA 15-3 and Carcinoembryogenic Antigen in Monitoring the Clinical Course of Patients with Metastatic Breast Cancer. Cancer Research 48:4107-4112

Tondini C, Hayes DF, Kufe D(1989): Circulating tumor markers in breast cancer. In Henderson IC (ed): Diagnosis and Therapy of Breast cancer. Philadelpuia, WB Saunders, 1989, pp 653-674.

Uterman G.(1989) The mysteries of lipoprotein (a). Science 1989; 17 Nov: 904-910

Virtanen,I.,Letno,VP.,L,Lehtonen,E.,Vartio,T.,Stenman,S.,Kurki,P.,Wager,O.,Smal l,J.V.,Dahl,D.&BAadley,R,A..(1981) Expression of intermediate filaments in cultured cells. J.Cell Sci.,50,45

W Yeo, WH Kwan, WY Lee, TWTLeung ,PWL Teo, W King, PJ Johnson(1996) Familial breast cancer in Hong Kong Chinese HKMJ 1996;:2:68-71

Waseem,A., Alexander,C.M., Steel,J.B. & Land,B.(1990a) Localization of the gene for human simple epithelial keratin 18 to chromosome 12 using polynerase chain reaction.Genomics,7,188-194

Waseem,A., Alexander,C.N., Steel,J.B. & Lane,B.(1990a) Embryonic simple epithelial keratins 8 and 18:chromosomal location cmphasizes difference from other keratin pairs. New Biol.,2,464-478

Weber,K. & Geisler,N. (1984) Intermediate filaments-from wool-keratins to neurofilament. A structural overview. In:A.J.Levine,G.F.van de Woulde,W.C.Topp,& J.D.Watson(eds.),Cancer cell, the transformed phenotype,pp.153-159,Cold Spring Harbor Laboratory, New York 1984.

Weber,K., Osborn,N., Noll,R., Wiklund.,B. & Luning,B.(1984) Tissure polypeptide antigen(TPA) is related to the nonepidermal keratins 8,18 and 19 typical of simple and nonsquamous epithelia:re-evaluation of a human tumor marker.EMBO J.,3,2707-2714

Wieczorek, A. J., G. Phyner and L. H. Block (1985). Isolation and characterisation of an RNA proteolipid complex associated with the malignant state in tumours. Proc. Nat. ACAD. Sci. USA 82, 3455-3459

Worman,H.J.(1990) Cellular intermediate filament network and their derangement in alcoholic hepatitis. Alcohol Clin.Exp.Res.,6789-804

Wright, L. C., D. R. Sullivan, M. Muller, M. Dyne, M. M. N. Tattersall and C. E. Mountford (1989). Elevated apolipoprotein (a) levels in cancer patients. Int. J. Cancer 43, 241-244.

WWK King ,YP Wang ,JTF Kau, SSC Chung, PJ Johnson, AKC Li (1996) Analysis of 346 Chinese patients with breast cancer HKMJ 1996:2:72-79